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IMPLANTATION CARCINOMA OF THE TUBAL MUCOSA SECONDARY TO CARCINOMA OF THE OVARY *

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It is the purpose of this paper to demonstrate changes in the mucosa of the fimbriae and ampulla of the Fallopian tubes of patients with ovarian carcinoma which may provide a fertile soil for the implantation and growth of cancer cells on its surface, and likewise to present the various stages in the actual implantation of cancer cells in these situations and the subsequent life history of the resulting secondary neoplasms. In addition I wish to emphasize that the pathogenesis, structure, form and life history of carcinomatous implants of ovarian origin on the tubal mucosa are the same as the pathogenesis, structure, form and life history of similar implants on the peritoneal serosa.

In 1934 the writer [1] published the results of a study of a series of carcinomas of the tubes and ovaries secondary to carcinoma of the body of the uterus. In that paper 9 cases of carcinoma of the tubal mucosa were reported. In 2 of these cases the carcinoma evidently reached the tubal mucosa by lymphatic permeation or metastasis from the uterine neoplasm. In the remaining 7 cases, 1 of which had also been reported in an earlier paper,[2] the tubal carcinomas were judged to have arisen from cancer cells that had escaped from the uterine tumor through the uterine ostium of the tube into its lumen and had become grafted on the tubal mucosa.

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The data supporting the implantation theory for the origin of the secondary tumors of the tubal mucosa in these 7 cases are: the tubal carcinomas were superficial in character and presented the appearance either of having arisen from the epithelium of the mucosa or of having been added to it; their histological structure was similar to that of many of the implantation carcinomas on the lining of lymph vessels and on the peritoneal serosa; in all but one instance they were not only multiple but varied in size, thus suggesting different ages; evidence was present in each instance indicating that cancer cells from the uterine growth could easily have escaped into the lumens of the tubes; clumps of cancer cells were found in the lumens of the tubes; on the other hand carcinoma was found in the lymphatics of these 7 tubes in only one instance (Case 11 of that paper [1]) and well may have arisen from the invasion of the fimbrial mucosa by carcinoma implanted on its surface.

Since the tubal carcinomas presented the histological picture either of arising from the mucosa or of having been added to it, the possibility of a multicentric origin for some or even all of the tubal tumors could not be excluded.

Newly formed tissue on the surface of the tubal mucosa with cancer cells enmeshed in it was found in only one instance (see Fig. 77 of previous paper [1]). The failure to find more evidence of newly formed tissue on the surface of this mucosa, as well as the failure to detect all the stages in the implantation of cancer cells on the tubal mucosa in this series of cases, aroused my curiosity and urged me to study further the implantation of cancer cells in this situation. This is the real incentive for the study of the problem of the present paper.

If cancer cells actually become implanted on the surface of the tubal mucosa, the secondary tumors should not only resemble carcinomatous implants in other situations but all stages in their pathogenesis must be demonstrated. Otherwise this theory cannot be accepted.

If cancer cells escaping from a uterine carcinoma into the lumen of the tube become implanted on its mucosa, cancer cells escaping from an ovarian carcinoma into the peritoneal cavity might become implanted on the mucosa of the fimbriae and ampulla of the tube. The fimbriae of the tubes are immersed in the ascitic fluid

frequently encountered in patients with ovarian carcinoma. Cancer cells are usually present in this fluid. We have abundant evidence that cancer cells floating about in ascitic fluid and in the lumens of lymph vessels and of the tubes may not only be alive but may live a long time and actually increase in numbers in these situations. It is natural to assume, therefore, that these cells sometimes might become implanted on the surface of the fimbrial mucosa and also on the surface of other portions of the tubal mucosa, just as they become implanted on the lining of lymph vessels and on the surface of the peritoneum when a fertile soil arises for their implantation and growth.

In the study of the life history of any secondary neoplasm we should realize that its growth and spread, irrespective of its pathogenesis, depend on its inherent traits and likewise on the "soil" and the structure of the organ in which it is located. We must anticipate that if carcinoma becomes implanted on the tubal mucosa it may invade the mucosal lymph vessels and in this way it may present histological findings that may be confused with those resulting from metastases to the tubal mucosa from the ovarian carcinoma by way of the lymphatics. A knowledge of the lymphatics of the tubal mucosa, therefore, is essential for the study of the life history of all carcinomas of this mucosa. In order to be able to study intelligently implantation carcinoma of the tubal mucosa secondary to ovarian carcinoma, it is necessary to appreciate the changes in this mucosa caused by carcinoma reaching it by other ways than implantation.

The Status of the Implantation of Cancer Cells on the Surface of the Tubal Mucosa

The origin of metastases from the implantation of cancer cells on epithelial surfaces has been and still is a debatable one. This subject is well presented by Willis [3] in his excellent monograph published in 1934. He states: "There are strong *prima facie* reasons why we must regard inoculation metastasis as an improbable event in most situations. The presence of a rich bacterial flora in skin and in alimentary and respiratory mucous membranes, the presence of digestive enzymes and mucus in the alimentary tract and of mucus in the respiratory tract, the certain enfeebled vitality of fragments of tumour detached from ulcerating growths, and

the absence of breaches in healthy epithelial surfaces — all these considerations should give pause to a too facile assumption of implantation metastasis, however plausible in explaining the conditions observed. Before adopting this explanation in any given case, it is essential to inquire whether the supposed implant tumours may not be either multiple primary growths or metastasis by other routes, lymphatic, transcoelomic or hemic."

In discussing the origin of inoculation metastasis in the female genital tract Willis states: "Since the cavities of the healthy uterus and tubes are bacteria-free, and since they contain no lytic secretions and but little mucus, tumour implantation in the tubal mucosa or the endometrium must be regarded as by no means improbable. In the vagina or vulva, on the contrary, the conditions must be highly unfavourable to successful implantation. The literature contains many records of possible or probable examples of tumour inoculation in the female genital tract. In reviewing these it is important to recognize that in this territory local metastasis via lymphatic and venous channels is a frequent event, and that many seeming instances of implantation may equally well be attributed to spread by these familiar routes. In a brief but valuable paper, Glendining [4] (1910) advanced evidence for the occurrence of tubal mucosal implantation of tumor cells wafted by the fimbriae into the tubes from the peritoneal cavity, but the observations did not permit conclusive proof. Kaufmann (p. 1061) observed distension of the tube by carcinomatous masses secondary to a mucoid gastric cancer, and attributed these to mucosal implantation in the tube. Sitzenfrey (cited by Kaufmann), Kundrat [5] (1906), Werner [6] (1914), Sampson [2] (1924), Offutt [7] (1932) and others have described specimens which strongly suggest retrograde passage of fragments of uterine cancer into the tubes with mucosal implantation in these organs." However, none of the above mentioned authors described the various stages in the implantation of these cells on the tubal mucosa, which is so essential for the acceptance of this theory.

In conjunction with one phase of the problem of the present paper Glendining's [4] contribution is a valuable one. He reported the results of a careful study of the tubes from two patients with carcinoma of the ovaries secondary to carcinoma of the stomach. In both instances he found cancer cells in the lumens of the tubes

and carcinoma in the mucosal lymphatics. He believed that cancer cells floating in the peritoneal cavity were swept into the lumens of the tubes by their fimbriae, became grafted in their mucosa and from there penetrated the subepithelial lymphatics and thus escaped into the lymph vessels of the mesosalpinx. In both instances the efferent lymph vessels of the ovaries were permeated by the growth. Glendining correctly concludes that one cannot entirely exclude the possibility of the origin of the tubal carcinoma from the ovarian tumors by lymphatic permeation.

I have studied the microphotographs published by Glendining [4] and believe that the carcinoma in the mucosal lymphatics in his cases probably came from the ovarian tumor by way of these vessels and not from the implantation of cancer cells on the tubal mucosa. I also believe that some of the clumps of cancer cells in the lumens of the tubes well may have reached this situation by growing out from the mucosal lymphatics through the overlying epithelium. This latter phenomenon I have frequently observed.

Even though Glendining's [4] interpretation of the sequence of events in the specimens studied by him may not be correct, nevertheless he has contributed a very interesting and stimulating theory, namely, that cancer cells floating about in the peritoneal cavity may be swept by the fimbriae into the lumen of the tube, become implanted on the tubal mucosa, and by their invasion of the underlying lymphatics gain access to the lymphatic circulation of their host.

The Implantation of Cancer Cells on the Peritoneum as a Guide in the Study of the Implantation of Similar Cells on the Tubal Mucosa

I have assumed in the study of the present problem, until proved otherwise, that the pathogenesis of the implantation of cancer cells on the tubal mucosa should not differ greatly from the pathogenesis of the implantation of similar cells on the peritoneum.

In 1914, Jones and Rous [8] published the results of a series of experiments in which Kieselguhr (finely ground diatomaceous earth) suspended in Ringer's solution was injected into the peritoneal cavity of mice, followed later by a suspension of mouse carcinoma cells. Implantations of carcinoma occurred at the site of the peritoneal injury caused by the Kieselguhr. On the other

hand, implants were not found in the peritoneal cavity of control animals injected only with cancer cells. Similar results were obtained by these workers when pieces of small glass rods and dead cancer cells were introduced into the peritoneal cavity of these animals prior to the injection of a suspension of the living cancer cells. They demonstrated that the localization of the peritoneal metastases was determined by the proliferation of the submesothelial connective tissue at the site of injury, which formed a supporting stroma for the living tissue cells subsequently introduced into the peritoneal cavity. They believed that dead cancer cells in contact with the serosa of human beings may cause changes favorable for the lodgement and growth of living cancer cells.

It was shown in a paper on implantation peritoneal carcinomatosis of ovarian origin, published by the writer [9] in 1931, that the implantation of the cancer cell is made possible by injury to the peritoneum causing a local reaction of its submesothelial tissues with a disappearance of the mesothelium over this area. The degree of this reaction varied greatly in individual cases. In some instances it was slight. In others strands or masses of granulation tissue arose on the surface of the serosa, forming sessile or pedunculated outgrowths of this newly formed tissue. Cancer cells were frequently found anchored to the peritoneum by fibrin at the site of injury and attached to or enmeshed in the strands and polypoid outgrowths of granulation tissue. On the other hand, similar peritoneal reactions without cancer cells attached to or enmeshed in them were occasionally seen, especially in the early cases of peritoneal carcinomatosis. It is evident that these areas of newly formed tissue, whether slight or exuberant, supply the soil on or in which cancer cells floating in the peritoneal cavity lodge and grow. I do not know the cause of the injury nor why it is local. Obviously cancer cells escaping into the peritoneal cavity in some way are responsible.

The form of the mature implant varies with the type of the initial reaction of the peritoneum to the injury, the attempted repair of this injury, the growth of the cancer cells and the age of the implant. As a result of these processes carcinoma becomes embedded in peritoneal scars, encapsulated on its surface, enmeshed in adhesions and in the organized polypoid outgrowths of granulation tissue or, like a surgical skin graft, grows on the sur-

face of the peritoneum without encapsulation. All stages in the development of these various types of peritoneal implants have been described by the writer.[9] We must, therefore, regard an *implant as a true neoplasm which may consist solely of cancer cells, but more often consists of both cancer cells and a stroma of newly formed tissue which is derived from its host.*

The implantation of cancer cells on the tubal mucosa cannot be accepted until all stages in the development of the various types of these secondary tumors in this situation have been demonstrated. Whether or not the various stages in the implantation of cancer cells in the two situations are the same will be shown.

The Life History of the Peritoneal Implants as a Guide in the Study of the Life History of Mucosal Implants of the Fallopian Tube

What happens to cancer cells implanted on the peritoneum? Some die, others apparently multiply very slowly and remain efficiently encapsulated by dense connective tissue for a long time. This latter phenomenon is very evident in some of the mature pedunculated polypoid implants. If the cancer cells of the implant are not encapsulated an opportunity for the dissemination of some of these cells from this focus into the peritoneal cavity is present.

We realize that many patients with peritoneal carcinomatosis do not die from the primary ovarian tumors but from intestinal obstruction caused by the invasion of the intestines by the carcinoma implanted on their surfaces. This is an indication of the marked invasive trait of the cancer cells in some of these implants. They invade the tissues of their host like a primary tumor. Carcinoma may be found in the lymphatics beyond the advancing margin of the growth (see Figs. 51 and 55 of previous paper [9]).

If cancer cells become implanted on the tubal mucosa we would expect that they might have a life history similar to cancer cells implanted on the serosa and that they might invade the tubal lymphatics and even reach the ovary through these channels. The latter phenomenon (which is not impossible) would cause a confusing picture.

In a recent paper by the writer [10] on the origin and significance of newly formed lymph vessels in carcinomatous peritoneal implants, it was demonstrated that in some instances newly formed

lymph vessels as well as newly formed blood vessels participate in the development of cancer-free granulation tissue arising on the surface of the peritoneum of patients with peritoneal carcinomatosis. This tissue forms the stroma of peritoneal implants. Newly formed lymph vessels were found also in the stroma of some of these implants. In a few instances conditions were encountered suggesting that the presence of these newly formed lymph vessels in the stroma of an implant might have permitted an earlier spread of the neoplasm into the lymphatic circulation of its host than would have occurred had these vessels not been present (see Figs. 86, 111, 112 and 113 of that paper [10]).

Since newly formed lymph vessels are sometimes present in newly formed tissue arising on the surface of the serosa of patients with peritoneal carcinomatosis, one would expect that carcinoma in preexisting lymphatics beneath the newly formed tissue might invade the newly formed lymph vessels. This condition has been encountered (see Figs. 129, 130 and 131 of previous paper [10]).

If cancer cells become implanted on the tubal mucosa one would expect that they might have a life history similar to cancer cells implanted on the serosa and that some or even all of the phenomena just described might be encountered in the study of mucosal implants.

The Lymphatics of the Mucosa of the Fimbriae and Ampulla of the Tube

In the study of carcinoma of the tubal mucosa secondary to carcinoma of the ovary I have been impressed with the great frequency of the location of these tumors in the distal portion of the tube and also with the presence of the growth in spaces in its mucosa which I believed might be lymph vessels. It appeared to me that in some instances the carcinomas in the mucosal lymphatics had not necessarily come from the primary ovarian growth through these channels but well may have reached their present situation from the spread of a secondary tumor of the tubal mucosa of other origin than lymphatic metastasis or permeation from the ovarian tumor. Irrespective of the method of origin of those secondary tumors from the ovarian growth, a knowledge of the lymphatic circulation of the mucosa of the tube, especially that of its distal portion, is essential in order to understand the patho-

genesis of some of these tumors and the possible subsequent life history of all of them.

In endeavoring to ascertain the significance of the various conditions encountered in the study of these secondary carcinomas of the tubal mucosa, I found that I was greatly handicapped by a lack of any conception of the lymphatic circulation of the mucosa of the tube. I therefore attempted to ascertain the distribution of the lymph vessels in the mucosa of the fimbriae of the tube and their relation to the lymphatics of the ampulla and to those of the mesosalpinx and of the ovary. Some of the results of these endeavors have recently been published.[11]

I have been unable to find in the literature an adequate description of the pattern of the lymphatics in the mucosa of the ampulla and fimbriae of the tube in human beings. Andersen,[12] however, has given an excellent description of the injected lymphatics of all layers of the ampulla of the tube of the sow. Owing to technical difficulties she was unable to inject the lymphatics of the mucosa of the infundibulum.

I have observed both in the mucosa and in the walls of the tubes from patients with carcinoma of either the uterus or the ovary, spaces that were filled with carcinoma as with an injection mass (see Figs. 10 and 11). I have also frequently seen irregular empty spaces in other portions of the tubal mucosa just described and also in the mucosa of many normal tubes which, in form and in situation, resembled the spaces filled with carcinoma. In these empty spaces an endothelium-like lining could be detected. I inferred that these carcinoma-filled and empty spaces were lymphatics. I gathered this material and compared these spaces with Andersen's [12] illustrations and descriptions of the injected lymphatics of the mucosa of the ampulla of the sow. I found that the judged lymphatics observed by me were similar, both in form and in distribution, to the injected lymphatics of the sow's tube shown by Andersen.

Since the mucosa of the fimbriae is a continuation of the mucosa of the ampulla through the abdominal ostium of the tube and has the same histological structure as the latter, the distribution of the lymphatics in these two situations should be similar. I studied carcinoma-filled and empty lymphatics of the fimbrial mucosa by comparing them with similar lymphatics in the mucosa of the

ampulla. I found that the pattern of the lymphatics in the two situations was the same.

For descriptive purposes the lymphatics of the ampullar and fimbrial mucosa may be divided into two plexuses; one situated in the mucosa at the base of and between the folds, and the other in the folds. Vessels from the plexus in the folds empty into the plexus at the base of the folds. Thus the lymphatics of one mucosal fold are united with those of adjacent folds. This general pattern prevails in all sizes and types of mucosal folds whether in the ampulla or fimbriae (see Figs. 2, 3 and 5). Since the lymphatics of the ampullar and fimbrial mucosa are true capillaries without valves a free circulation of the lymph is assured in all directions in the plexuses.

I was able to demonstrate that the lymphatics at the base of and between the mucosal folds of the fimbriae about the ostium of the tube are continuous with similar lymphatics of the distal portions of the ampulla. Also when a fold of fimbrial mucosa is a continuation of a longitudinal fold of the ampulla the lymphatics in the two folds as well as at their bases are continuous. This well may account for the frequent presence of carcinoma both in the lymphatics of the fimbrial mucosa and in those of the adjacent ampullar mucosa of the same tube.

Evidence was found indicating that the mucosal lymphatics of the fimbriae drain into vessels in the wall of the infundibulum and also into vessels in the mesosalpinx beneath the ovarian fimbriae just as the lymphatics of the ampullar mucosa drain into vessels penetrating the wall of the tube beneath it.

I was unable to detect any evidence of an anastomosis between the lymphatics of the fimbrial mucosa and the subserosal lymph vessels at the mucoserosal junction even in fimbriae in which the mucosal lymph vessels were filled with carcinoma. Andersen [12] has shown that the subserous lymph capillaries of the distal portion of the ampulla of the sow's tube are few and inconspicuous as compared with the rich lymphatic plexus of the mucosa. It is my impression that the same is true in the tubes of human beings.

An anastomosis between lymph vessels coming from the hilum of the tubal pole of the ovary and the lymphatics of the adjacent ovarian fimbriae may well exist but was not positively demonstrated by me. The suggestion of the possibility of such an

anastomosis was found in only two of my specimens. Polano [13] found a lymphatic loop in the ovarian fimbriae by injecting the lymphatics of the ovary. Kroemer,[14] by injecting the tubal lymphatics, found communicating branches running along the ovarian fimbriae.

Andersen demonstrated that in the sow (Fig. 1 of her paper [12]) the lymph vessels from all portions of the tube, including the fimbriae, converge to form the subovarian lymph plexus which also receives lymph vessels from the ovary and the uterus. She refers in her paper to the contributions of Poirier, Bruhns, Bartels, and Poirier and Cuneo, indicating that lymph vessels from the tube unite with similar vessels from the ovary and uterus.

Pellé and Pellé [15] (1931) by first injecting the lymphatics of the tubes with Paris blue and then those of the ovary or uterus with cadmium yellow observed that the blue colored lymph vessels coming from the tubes soon became green, thus indicating an early communication between the lymph vessels of the tube and those of the ovary and uterus. The anatomical observations just mentioned may be further confirmed by the abundant evidence in pathological studies that carcinoma may spread from either the uterus or the ovary to the tube, by way of the lymph vessels (see Figs. 10 and 11).

A study of the lymphatics of the fimbrial and ampullar mucosa indicates that they are very abundant and that while the larger ones are usually more centrally situated in the mucosal folds than the blood vessels, a very superficial, almost subepithelial, portion of the mucosal plexus may sometimes be detected (see Figs. 7 and 8). The mucosal lymphatics of the ampulla apparently are larger, more abundant, and possibly nearer the surface than the subserosal lymphatics.

The following conditions have been demonstrated in previous papers by the writer: the invasion of the lymphatics of the tubal wall by carcinoma implanted on its serosal surface; the presence of newly formed lymph vessels in cancer-free granulation tissue arising on the serosa of the ampulla of the tubes of patients with peritoneal carcinomatosis; the extension of carcinoma in the lymphatics of the tube into the lymph vessels of newly formed tissue on its serosa, and the presence of newly formed lymph vessels in the stroma of serosal tubal implants. If granulation tissue

and carcinomatous implants occur on the fimbrial and ampullar mucosa which has a richer and possibly more accessible lymphatic plexus than the ampullar serosa, one would anticipate that some or all of the phenomena just described might be found in the study of mucosal newly formed tissue and implants.

Material and Methods of Study

The material for this study was obtained from the gynecological service of the Albany Hospital. The technical part of the work was carried on in the pathological laboratory of that hospital and the Albany Medical College. On the other hand, the greater part of the time spent by me in the actual study of the problem, including the microscopic examination of sections and writing, was done during "vacations" away from routine professional responsibilities.

From 1921 to 1936, inclusive, 148 patients with ovarian carcinoma were operated upon in the gynecological service of the Albany Hospital. Judged secondary carcinoma of 1 or both tubes was encountered in 23 of these patients. Since carcinoma was found in both tubes in 9 cases, 32 tubes with secondary carcinoma of their mucosa, multiple in many instances, were available for this study.

During the earlier and major part of this period I was greatly interested in peritoneal carcinomatosis. After the removal of the uterus, tubes and ovaries from patients with ovarian carcinoma, the tubes with serosal implants were severed from the ovary before blocking them. Thus an opportunity to study critically the possible extension of the ovarian carcinoma into the lymph vessels of the mesosalpinx was lost in these cases. During that period the only portions of the tubes that were carefully examined microscopically were those with obvious carcinomatous implants on their serosa. The fimbriae were not sectioned unless they grossly presented the appearance of malignancy. If the tubes appeared normal usually only one block was cut from each tube. From the standpoint of the present problem this method of studying the greater portion of this material was incomplete and gives no statistical data in determining the frequency of implants on the tubal mucosa.

However, in recent years special attention has been paid to the

fimbriae of the tubes of patients with ovarian carcinoma. They are inspected at the time of the operation. Great care is exercised that they shall not be traumatized during the removal of the pelvic organs. Should they appear abnormal, careful drawings are made of them. All are studied microscopically.

All surgical specimens are described in the pathological laboratory, tissue is removed, fixed in Zenker's solution, embedded in paraffin, cut, stained and examined as a routine procedure. This routine examination of surgical specimens is of greatest value for records and diagnostic purposes but is not sufficient for the intensive study of special problems. Through the courtesy of Dr. Victor C. Jacobsen, the former director of the laboratory, and of Dr. Arthur W. Wright, the present director, I have retained specimens or portions of specimens in which I have been specially interested, saving for them sufficient material for routine laboratory records.

All of the tissue from which the sections shown in this paper were made was fixed in 10 per cent formalin and embedded in celloidin. This procedure lessens unequal tissue shrinkage. As a result fewer distortions and exaggerated tissue spaces occur than in paraffin embedding after formalin fixation. The sections were stained with hematoxylin and eosin.

I block the tissue myself. The embedding of this tissue, section cutting and staining are entrusted to a well trained technician who studies the gross specimen with me when the blocks are made and understands how the blocks are to be mounted and what to look for in each block.

Serial sections have been extensively employed in recent years and are sometimes indispensable. In some instances all sections of a portion of a block are stained and studied but more often sections are stained and mounted at intervals, the latter depending on the judged importance of the tissue in a given instance. All sections, however, are saved for further study if this is indicated. Frequently a section is stained and examined while trimming the block in order to ascertain if serial sections are indicated. Since complete serial sections are time consuming and frequently unfruitful they are not made unless they are indicated by a test section.

Microphotographs have been extensively used in the study of this and other problems. I find that they are of the greatest value in the comparative study of conditions present in different sections

from the same or different tubes. The main object in publishing a large number of microphotographs is that others may study them as I have, form their own conclusions and compare this material with their own. Since I desire to demonstrate that the pathogenesis, structure, form and life history of carcinomatous implants on the tubal mucosa are the same as the pathogenesis, structure, form and life history of similar implants on the peritoneum, I have chosen to publish microphotographs of serosal implants present in some of these cases. This important feature has increased the number of illustrations.

Case Reports

The following 13 cases have been selected from the 23 of carcinoma of the tubal mucosa secondary to carcinoma of the ovary in order not only to report briefly the most important cases with implants on the tubal mucosa, but also, for the sake of comparison, to include 4 others in which the secondary mucosal carcinomas were obviously of other origin than implantation. The latter cases are presented first. The main purpose of the report of these 13 cases is to supplement the illustrations and their legends, which constitute the most important part of this paper since they present my observations and interpretations better than any written description alone.

Case 1. Carcinoma of the ampullar and fimbrial mucosa of the left tube secondary to adenocarcinoma of the left ovary, which had invaded the mesosalpinx, including its lymph vessels, and thus may have gained access to the tubal lymphatics. The patient, A. H. No. 93646, nulliparous and aged 46 years, was operated upon Nov. 9, 1923. A large tumor of the left ovary was found, which was wedged in the pelvis and extended upward nearly to the level of the umbilicus. There was no gross evidence of peritoneal carcinomatosis. The right tube and ovary appeared normal. Both tubes and ovaries and the uterus were removed. The patient survived the operation but ultimately died of carcinoma. The evidence, found in the study of this specimen, indicates that the carcinoma of the tubal mucosa arose from the ovarian tumor through the lymph vessels (see Figs. 11 to 14 inclusive). Carcinoma was found in the right tube only in the form of serosal implants and in the right ovary in the form of lymphatic permeations

and emboli. The portal of entry of the carcinoma in the lymph vessels of the right ovary was not determined.

CASE 2. Carcinoma of the mucosa of the left tube including its fimbriae, secondary to adenocarcinoma of the left ovary, with evidence that the tubal carcinoma was derived from the ovarian tumor through the lymph vessels. The patient, A. H. No. 6447–29, parous and aged 56 years, was operated upon Sept. 16, 1929. The uterus contained multiple leiomyomas. A tumor, about 8 cm. in diameter, had replaced the left ovary. The right ovary and tube appeared normal. Metastases were present in the posterior cul-de-sac but none were detected in the omentum. Both tubes and ovaries and the uterus were removed. The patient survived the operation but eventually died of carcinoma. Carcinoma can be seen extending from the ovarian tumor in lymph vessels which accompany the blood vessels in the hilum of the ovary (Fig. 15) and also in the lymph vessels of the mesosalpinx and tubal wall. The carcinoma of the tubal mucosa, including its fimbriae, evidently arose from this source (see Figs. 16 and 17).

CASE 3. Carcinoma of the mucosa of the uterine portion of the left tube and also of its isthmus and ampulla secondary to an advanced adenocarcinoma of the ovary which had invaded the left cornu of the uterus and the proximal portion of the tube. The patient, A. H. No. 5466–34, parous and aged 56 years, was operated upon July 20, 1934. Bilateral ovarian carcinoma and an extensive peritoneal carcinomatosis including metastases to the omentum were found. The uterus contained multiple leiomyomas. The distal ends of both tubes appeared normal. The ovarian tumors were adherent to the uterus and the proximal portion of both tubes. Both tubes and ovaries and the uterus were removed. The patient survived the operation but eventually died of carcinoma. The left tube and uterine cornu with the portion of the ovarian tumor attached to them were carefully studied. By continuous extension the ovarian carcinoma had invaded the left uterine cornu and the proximal portion of the tube including its mesosalpinx (see Figs. 18 to 20 inclusive).

CASE 4. Carcinoma of the fimbriae of the left tube secondary to adenocarcinoma of the ovary, due to continuous extension of the primary ovarian growth. The patient, A. H. No. 56392, nulliparous and aged 52, was operated upon Sept. 20, 1922. A carcinoma of

the left ovary and a peritoneal carcinomatosis including metastases to the omentum were found. The opposite tube and ovary appeared normal. Both tubes and ovaries and the uterus were removed and a portion of the omentum was resected. The patient recovered from the operation but eventually died of carcinoma. The carcinoma of the ovary had invaded the fimbriae of the left tube (see Figs. 21 and 22). Carcinoma was not found in the right tube and ovary.

CASE 5. Multiple papillary carcinoma of the tubal mucosa secondary to carcinoma of the ovary, due to the implantation of cancer cells on the mucosa of the tube which primarily had escaped from the carcinoma of the ovarian cyst into the lumen of the tube through its patent ostium (tubo-ovarian cyst). The patient, A. H. No. 2960–31, nulliparous, aged 50 years and 2 years past the menopause, had had the appendix and right tube and ovary removed at the age of 20 years. At the second operation, April 24, 1931, a tubo-ovarian cyst on the left side was found. Peritoneal carcinomatosis was not evident. The left tube and ovary and the uterus were removed. The patient recovered from the operation but eventually died of carcinoma.

The specimen was hardened in formalin before incising the tubo-ovarian cyst (see Fig. 23). The series of sections of the tube in this case are sufficiently complete to enable one to study all stages in the pathogenesis of the implants on the tubal mucosa.

The following observations were made in the study of this tubo-ovarian cyst:

1. Changes in the tubal mucosa favorable for the implantation of cancer cells which had escaped into the lumen of the tube from the carcinomas of the ovarian cyst and which were shown also to have escaped from the secondary carcinomas of the tubal mucosa. These changes in the tubal mucosa are: (*a*) sessile and pedunculated polypoid outgrowths of granulation tissue on the surface of the tubal mucosa (see Fig. 27); and (*b*) local reactions of the subepithelial tissues of the mucosa, manifested by a proliferation of the tissue cells and associated with either a partial or a complete loss of the overlying epithelium.

2. Cancer cells attached to and becoming embedded in granulation tissue on the tubal mucosa (see Figs. 28 and 30). Cancer cells, splinted by fibrin, on the areas of the mucosa with a local

reaction of the subepithelial tissues and a loss of the overlying epithelium (see Figs. 35, 36 and 37).

3. Neoplasms consisting of carcinoma embedded in or growing on the surface of polypoid newly formed tissue which had arisen on the surface of the tubal mucosa (see Figs. 29 and 31). Carcinoma replacing portions of the tubal epithelium as though grafted in it (see Figs. 32, 33, 34 and 35).

4. All stages in the development and growth of these types of mucosal implantation metastases, which are demonstrated in Figures 28 to 41 inclusive.

It is evident, therefore, that the pathogenesis, structure and form of the carcinomatous implants on the tubal mucosa in this case are the same as the pathogenesis, structure and form of similar implants on the serosa of patients with peritoneal carcinomatosis. The ovary was not studied as thoroughly as the tube. However, it appears that the pathogenesis of the judged secondary carcinomas of the ovarian cyst is the same as the pathogenesis of the carcinomas of the tubal mucosa.

CASE 6. Carcinoma of the fimbrial mucosa of both tubes secondary to adenocarcinoma of the ovaries, due to the continuous extension of the primary tumors to the fimbriae with replacement of portions of their mucosa. Multiple carcinomas of the mucosa of the fimbriae of the left tube due to the implantation of cancer cells on its surface. Multiple carcinomas of the mucosa of the ampulla of the right tube due to the implantation of cancer cells on its surface, which were disseminated into the lumen of that tube from the portion of the ovarian growth that had replaced the fimbriae and had extended through the abdominal ostium of the tube into the lumen of the ampulla. The patient, A. H. No. 7062–32, nulliparous and aged 73 years, was operated upon Oct. 5, 1932. Carcinoma of both ovaries and an extensive peritoneal carcinomatosis including metastases to the omentum were found. Both tubes and ovaries and the uterus and an epiploical appendage were removed. The patient survived the operation, but eventually died of carcinoma.

Two types of mucosal implants were found in this case. One type consists of polypoid newly formed tissue, which had arisen on the surface of the fimbrial and ampullar mucosa, with carcinoma embedded in it or growing on its surface (see Figs. 46, 47, 53 and 54). Various stages in the pathogenesis of these polypoid implants

were observed (see Figs. 47 and 54). In the second type carcinoma has replaced the epithelium of portions of the mucosal folds of the ampulla and presents the appearance of having been grafted in that epithelium (see Figs. 55 to 58 inclusive). The early stages in the pathogenesis of this latter type of implant, so well shown in the previous case, were not detected in the study of the tubes from the present case.

CASE 7. Carcinoma of the mucosa of the fimbriae of both tubes secondary to adenocarcinoma of the ovaries, due to the implantation of cancer cells in granulation tissue arising on the surface of the fimbrial mucosa. The patient, A. H. No. 3679–35, nulliparous and aged 41 years, was operated upon April 27, 1935. Carcinoma of both ovaries and an extensive peritoneal carcinomatosis, including metastases to the omentum, were found. Both tubes and ovaries and the uterus were removed (see Fig. 59). The patient survived the operation and had deep X-rays, but eventually died of carcinoma. Three implants, of the polypoid type, were found attached to the fimbrial mucosa of the right tube (see Figs. 63 and 64). Two of these implants are mature (see Figs. 66 and 68). The third implant presents an early stage in the development of this type of implant, namely, the embedding of cancer cells in the tuft of a pedunculated polypoid outgrowth of granulation tissue which had arisen on the surface of a mucosal fold (see Figs. 65 and 67). Only one implant was present on the mucosa of the fimbriae of the right tube. It is mature and of the pedunculated polypoid type (see Figs. 69 and 70). Newly formed lymph vessels are present in its pedicle in close proximity to the carcinoma of the body of the implant (see Fig. 71). Carcinoma was not found in the mucosa of the ampulla nor in the lymph vessels of any portion of the tubes. The histological structure of these mucosal implants is the same as the structure of similar serosal implants from the same patient shown in Figures 50, 51, 52, 58 and 60 of a previous paper.[10]

CASE 8. Multiple carcinomas of the mucosa of both tubes, including their fimbriae, secondary to adenocarcinoma of both ovaries. The carcinomas of the right tube arose from a continuous extension of the ovarian growth invading the fimbriae and also from the implantation of cancer cells on the mucosa of the fimbriae and the ampulla. The carcinomas of the left tube arose from lymphatic metastasis and permeation from the ovarian growth,

and also from the implantation of cancer cells in granulation tissue arising on the fimbrial mucosa. The patient, A. H. No. 9895, parous and aged 47 years, was operated upon Sept. 21, 1935. Carcinoma of both ovaries and an extensive peritoneal carcinomatosis including metastases to the omentum and the umbilicus were found. Both tubes and ovaries and the uterus were removed and the umbilicus was excised. The patient had deep X-ray treatments following the operation, but eventually died of carcinoma. Three secondary carcinomas of the right tube were found, one due to an extension of the ovarian carcinoma invading the ovarian fimbriae (see Fig. 85), the second a polypoid implant on the mucosa of the fimbriae (see Fig. 86), and the third a judged early implant on the mucosa of the ampulla (see Fig. 77). Carcinoma was not found in the lymph vessels of this tube. The carcinomas of the fimbriae of the left tube arose both from lymphatic metastasis and permeation from the ovarian carcinoma (see Figs. 87 to 91 inclusive), and also from the implantation of cancer cells in granulation tissue which had developed on the surface of its mucosa (see Fig. 93). The carcinoma of the ampullar mucosa of this tube (Fig. 92), arose from lymphatic metastasis and permeation. For a further consideration of the findings in this case see Figures 73 to 96 inclusive.

Case 9. Carcinoma of both the fimbrial and the ampullar mucosa of the right tube and of the ampullar mucosa of the left tube secondary to carcinoma of both ovaries. The secondary tubal carcinomas in all three situations were judged to be of probable implantation origin with subsequent invasion of the mucosal lymph vessels. The patient, A. H. No. 7467–30, parous and aged 42 years, was operated upon Oct. 15, 1930. Carcinoma of both ovaries and an extensive peritoneal carcinomatosis including metastases to the omentum were found. Both tubes and ovaries, the uterus and an epiploic appendage were removed. The patient recovered from the operation but eventually died of carcinoma. Many peritoneal implants from this patient have been studied and some of these have been described in two previous papers [9, 10] (see Case 3 and Case 5 of these papers). The implants on the epiploic appendage were especially instructive. They were of various types and showed all stages in their development. Carcinoma was not found, in spite of a careful search, in the lymph vessels of the appendage. The im-

plants on the serosa of the tubes were similar to those on the appendage. All stages in their development could also be seen in this situation. However, carcinoma was found in the lymph vessels beneath some of the larger serosal implants on the right tube. It was judged that the carcinoma in these lymph vessels might have come from the spread of the carcinoma in the implants above them rather than primarily from the ovarian growth, even though the carcinoma in the ovary had invaded the lymph vessels of that organ (see Figs. 85 to 95 inclusive of a previous paper,[10] and also Fig. 101 of the present paper).

Longitudinal sections of the fimbriae of the right tube present an interesting and difficult problem. The lymphatic vessels of the fimbrial folds at the right in Figure 102 are filled with carcinoma, as with an injection mass evidently coming from the extension of the growth in lymph vessels in the base of these folds and between them. Carcinoma is not present in the lymph vessels of the fimbrial mucosa at the left. The tips of the mucosal folds in the center of this section are fused together by newly formed tissue of various ages containing carcinoma. On the other hand, the lymph vessels in the base and between these fused folds are not distended with carcinoma as in the folds at the right. A conglomerate tumor consisting of fused polypoid outgrowths of newly formed tissue of various ages containing carcinoma is attached by a short pedicle to the fused portion of the folds. It is judged that this conglomerate tumor has arisen from the fusion of polypoid implants (see Fig. 107 and its legend). The picture here is similar if not identical with the cluster of implants on the serosa of the same tube shown in Figure 101.

To the right of the base of the conglomerate tumor, just described (Fig. 107), is a similar tumor consisting of the tips of mucosal folds which are united by newly formed tissue containing carcinoma. Very early granulation tissue "f," with small clumps of cancer cells embedded in it, apparently is of the same age as outgrowth "a." Beneath this tissue is older newly formed tissue containing a larger amount of carcinoma. The latter tissue has arisen from adjacent mucosal folds. The conditions shown here easily could have resulted from the fusion of newly formed tissue similar to that arising from the mucosal fold in Figure 106. Cancer cells "g" are shown becoming implanted on the tip of newly formed

tissue arising from the fimbrial mucosa. This is a miniature repetition of the process shown in Figure 106. I am unable to decide whether the structure marked "e" is a mature polypoid implant or the tip of a fold infiltrated with carcinoma. Its origin was missed in the incomplete series of sections. A cross section of a mucosal fold "h" (easily recognized as such on careful study under higher magnification) shows carcinoma in a central large lymph vessel which apparently is continuous with the growth in the judged smaller peripheral lymph vessels of the fold. Beneath this is another portion of the same fold which is infiltrated with carcinoma, some of which is in lymph vessels. It is impossible to determine whether the picture shown in this field represents the invasion of the folds, including their lymph vessels, by carcinoma implanted in newly formed tissue on their surfaces (see arrows) and the subsequent extension of the growth in lymph vessels, or the spread of carcinoma in lymph vessels into the tissues of the folds and thence into the newly formed tissue arising on the folds.

We have positively demonstrated in the study of these conglomerate tumors in this and other sections of this series that cancer cells have become implanted in granulation tissue arising on the mucosal folds of the fimbriae of this tube and that when this tissue arises on adjacent folds these newly formed tissues become fused. We have also shown that the implanted cancer cells have grown in these newly formed tissues as in similar tissues arising both on the serosa and on the mucosa of other cases. We may infer that the carcinoma in the older judged implants has invaded the folds beneath them and even may have penetrated the lymphatics of these folds. In this way the conditions found in the study of the fimbriae of this tube may be accounted for. However plausible this explanation appears to me I fully realize that it may not be conclusive in explaining the entire picture found in these fimbriae since the growth in the ovary had invaded the lymph vessels of that organ. Carcinoma in the ovarian lymph vessels might have spread to the lymph vessels of the fimbrial folds and from this source might have invaded the newly formed tissue arising on the folds in which cancer cells in the ascitic fluid became implanted.

Clumps of cancer cells similar to those present in the ascitic fluid are present in the lumens of both tubes (see Figs. 108, 109 and 110). In both tubes carcinoma has not only replaced portions

of the tubal epithelium but may have invaded the mucosal lymph vessels (see Figs. 110 to 116 inclusive). In all instances where carcinoma is present in lymph vessels beneath either a serosal or a mucosal metastasis resembling an implant, both the source of the carcinoma in the lymph vessels and the pathogenesis of the metastasis are debatable. If carcinoma implanted on the tubal serosa sometimes invades the underlying lymph vessels it is rational to believe that carcinoma implanted on the tubal mucosa sometimes might invade the mucosal lymph vessels, especially since these lymph vessels are more abundant and possibly more accessible than those of the serosa.

Case 10. Carcinoma of the mucosa of the ampulla of the tube secondary to carcinoma of the ovary, probably due to the implantation of cancer cells in granulation tissue arising on the surface of the mucosa. The patient, A. H. No. 92806, nulliparous and aged 41 years, was operated upon Sept. 29, 1923. Carcinoma of both ovaries and an extensive peritoneal carcinomatosis including metastases to the omentum were found. Both tubes and ovaries and the uterus were removed. The patient survived the operation, but eventually died of carcinoma. The ovarian carcinomas were small and apparently arose from the surface epithelium of the ovaries. The pathogenesis of the peritoneal implants in this case have been described (in Case 4 of a previous paper [9]). Two metastases were found attached to the mucosa of one tube. They are almost exact duplicates of the polypoid implants present on the serosa of the opposite tube (see Figs. 117 to 120 inclusive). Carcinoma was not found in the lymph vessels of either the ovaries or the tubes but was present in the lumens of both tubes, the fimbriae of which appeared normal. In a few situations carcinoma was found in the lymph vessels beneath some of the implants on the parietal peritoneum and also beneath the implant attached to the serosa of the mesosalpinx shown in Figure 121. Circumstantial evidence indicates that the metastases in the mucosa of the tube arose from the embedding of cancer cells, present in the lumen of the tube, in granulation tissue which had developed on the surface of the tubal mucosa just as similar implantations arise on the surface of the serosa.

Case 11. Carcinoma of the mucosa of the fimbriae and of the ampulla of the right tube secondary to carcinoma of the ovary

which well may have arisen from the implantation of cancer cells on the tubal mucosa. Carcinomas of the mucosa of the fimbriae and of the ampulla of the left tube which are so far advanced that it is impossible to determine the pathogenesis of the initial tubal tumor. The patient, A. H. No. 8617–33, parous and aged 53 years, was operated upon Nov. 27, 1933. Carcinoma of both ovaries and an extensive peritoneal carcinomatosis including metastases to the omentum were found. Both tubes and ovaries, the uterus and two epiploical appendages were removed. The patient survived the operation but eventually died of carcinoma. The study of the polypoid serosal implants is most instructive as a possible aid in determining the pathogenesis of the mucosal metastases of the right tube. Multiple polypoid implants are present on one epiploic appendage without any evidence of carcinoma in the preexisting tissues of the appendage (see Figs. 122 and 123). In another epiploic appendage, with similar but apparently older polypoid implants on its surface, carcinoma is present in the lymph vessels of the appendage with positive proof that it is continuous with the carcinoma in an implant which had invaded the tissues of the appendage beneath it and had thus penetrated the lymphatics (see Figs. 124 to 130 inclusive). Similar dual phenomena were observed in similar polypoid implants on the serosa of the right tube and mesosalpinx (see Figs. 131 to 136 inclusive). The metastasis of the fimbrial mucosa of the right tube (Fig. 138) consists of a pedunculated polypoid tumor of newly formed tissue, with carcinoma embedded in it, which is attached by a pedicle to a mucosal fold of the fimbriae. The histological structure of this metastasis is similar to that of the serosal implants just described. Carcinoma is present in the mucosal fold and in its lymph vessels beneath the attachment of the pedicle of the polypoid tumor to the fold. The carcinoma in the polypoid tumor is apparently continuous with the carcinoma of the mucosal fold (see Fig. 139). Recent newly formed tissue with cancer cells becoming embedded in it (see Figs. 138 and 142) has arisen on the surface of this polypoid tumor. This phenomenon frequently occurs in both serosal and judged mucosal implants and is one way in which these tumors increase in size. The carcinoma, in the lymph vessels of the fimbriae, occurred only in those of the mucosal fold beneath the polypoid tumor and of nearby folds. Carcinoma was not found in the lymph

vessels of the distal portion of the mesosalpinx. I believe that this polypoid tumor on the fimbrial mucosa is an implant and that the carcinoma in it has invaded the underlying lymph vessels just as the carcinoma in similar implants on the serosa, from the same patient, have invaded the lymph vessels beneath them.

A large pedunculated polypoid mucosal metastasis is present in the ampulla of the right tube (see Fig. 144). This is similar to the preceding one and has grown by the attempted implantation on its surface of single clumps and collections of clumps of cancer cells which had migrated into the lumen of the ampulla of the tube through its abdominal ostium (see Figs. 137 and 143). The structure, growth and fate of this conglomerate tumor is fully described in the legend of Figure 144. In Figure 145 is shown the judged pedicle "a" of this polypoid tumor. The pedicle which appears in cross section consists of newly formed tissue containing blood vessels best seen under higher magnification. It has the characteristic structure of the pedicles of other mucosal polypoid implants shown in this paper (see Figs. 67 and 68). Although it was followed through several sections, its attachment to the tumor above it could not be detected (series of sections not complete). The small size of the blood vessels in this pedicle would easily account for the necrosis of a large portion of the tumor. Lymph vessels cannot be detected in the pedicle. However, they may be present since they occur in similar pedicles. Carcinoma was not found in any of the sections of this pedicle. Cancer cells might, at one time, have escaped from the tumor through lymph vessels in the pedicle into the lymph vessels of the mucosa from which the newly formed tissue of the tumor, including its pedicle, arose. In Figure 146 is shown a portion "a" of the attachment of the pedicle to a mucosal fold and carcinoma "b" in a lymph vessel of the mucosal fold in close proximity to the origin of the pedicle. An embolus of cancer cells "c" appears in a lymph vessel of a mucosal fold adjacent to the previous fold. The lymph vessels, in which the carcinoma is situated, are in adjacent secondary folds of the same primary mucosal fold. Therefore cancer cells might easily migrate from one secondary fold to the other. Carcinoma was not found in any other lymph vessels of the ampullar mucosa or in those of the tubal wall except beneath the attachment of serosal implant "g" of Figure 144 (see also Figs. 132 to 136 inclusive). Carcinoma

in a lymph vessel of the mesosalpinx was found in only one section. It appeared as an embolus in a lymph vessel of a portion of the mesosalpinx near the attachment of serosal implant "g" of Figure 144, and could easily have come from this source. Why should carcinoma be found only in lymph vessels of the ampullar mucosa in close proximity to the origin of the judged pedicle of the mucosal metastasis shown in Figure 144, unless the carcinoma in one of the two situations was primarily derived from the other? The histological structure of the nucleus of this mucosal metastasis is the same as the structure of serosal implants from the same patient without and with evidence of the spread of the carcinoma in the implants through their pedicles into the lymph vessels of their host. I believe that both the origin and the subsequent life history of the serosal and mucosal metastases just described are the same.

Two small carcinomas of the ampullar mucosa of the right tube were encountered which presented the appearance of having arisen either from the tubal epithelium or from the implantation of cancer cells. Many of these cells were floating about in the lumen of the tube. One of these tumors is shown in Figure 150. I believe that the pathogenesis of such an implant is demonstrated in Figures 148 and 149.

When carcinoma is found in lymph vessels in close proximity to a secondary carcinoma one must always consider the possibility of its origin from the primary growth by metastasis or permeation through these channels. I was unable to exclude the invasion of the ovarian lymph vessels by the ovarian carcinoma. Such an incident, which frequently happens, would not interfere with the implantation of cancer cells both on the serosa and on the mucosa of the tube with the subsequent invasion of the underlying lymph vessels by the secondary carcinoma of the implants.

Secondary carcinoma of the mucosa of the fimbriae and adjacent ampulla of the left tube was found with evidence that the tumor in these situations had spread in the mucosal lymph vessels. It was also present in the lymph vessels of the mesosalpinx. The growth in the left tube was so far advanced that it was impossible to determine either the site or the pathogenesis of the initial secondary carcinoma in this situation.

Since the tubal mucosal lymph plexus is much richer than the serosal plexus and just as accessible as the latter, one might antici-

pate that carcinoma implanted on the mucosa would gain access to the underlying lymph vessels more readily than when implanted on the serosa.

CASE 12. Carcinoma of the mucosa of the fimbriae of both tubes secondary to carcinoma of the right ovary. The carcinoma of the fimbriae of the right tube was probably due to lymphatic metastasis or permeation from the ovarian carcinoma. Some and possibly all of the carcinomas of the fimbriae of the left tube were due to implantation. The patient, A. H. No. 1130–26, parous and aged 49 years, was operated upon July 7, 1926. A large carcinomatous cyst of the right ovary and an extensive peritoneal carcinomatosis including metastases to the omentum were found. Both tubes and ovaries, the entire uterus, appendix and an epiploic appendage were removed. A portion of the omentum was excised. The patient recovered from the operation, was temporarily improved, but eventually died of carcinoma.

A hydrosalpinx was present on the right side with a portion of the ovarian fimbriae sticking out from its distended closed end like a sore thumb from a closed fist. The lymphatics of this portion of the fimbriae are filled with carcinoma as with an injection mass (see Fig. 151). Carcinoma was not found in any other portion of this tube.

Multiple carcinomatous implants are present on the surface of the left ovary; apparently one of these had invaded the ovary (see Fig. 152). The fimbriae of the left tube appeared swollen. Both serosal and mucosal implants can be seen on this portion of the tube (see Fig. 154). Carcinoma apparently arising in the fimbriae about the abdominal ostium of the tube has invaded the fimbrial and ampullar mucosa including the lymph vessels. For evidence that this carcinoma may primarily have been of implantation origin with subsequent invasion of the mucosal lymph vessels, see Figures 151 to 156 inclusive.

CASE 13. Carcinoma of the mucosa of the fimbriae and ampulla about the abdominal ostium of the left tube secondary to carcinoma of the ovary. Some and possibly all of the carcinomas of the fimbrial mucosa are of implantation origin. The patient, A. H. No. 856618, nulliparous and aged 53 years, was operated upon July 22, 1922. Carcinoma of both ovaries and an extensive peritoneal carcinomatosis including metastases to the omentum were found.

Both tubes and ovaries and the uterus, the appendix and two epiploical appendages were removed. The patient recovered from the operation, had deep X-ray treatments, was temporarily improved, but eventually died of carcinoma. The peritoneal implants were carefully studied. The results of some of these studies have been presented in a previous paper [9] (see Case 5 of that paper). Grossly the fimbriae of the left tube appear swollen and retracted (see Fig. 157). Implants are present on a mucosal fold of the fimbriae about a carcinoma which apparently started in or on the mucosal folds of the fimbriae around the abdominal ostium of the tube and had invaded the folds of the fimbriae and adjacent ampulla including their lymphatics (see Figs. 161, 162 and 163). For evidence that this carcinoma primarily may have been of implantation origin with subsequent invasion of the lymph vessels, see Figures 158 to 163 inclusive. Carcinoma was not found in the mucosa of the opposite tube.

The Pathogenesis of Carcinoma of the Tubal Mucosa Secondary to Carcinoma of the Ovary

Carcinoma of the tubal mucosa secondary to carcinoma of the ovary was encountered in 23 patients. Since carcinoma was found in both tubes in 9 cases and in 1 tube only in the others, 32 tubes with secondary carcinoma of the mucosa, multiple in many instances, were studied. Carcinoma was detected in the fimbrial mucosa in 25 of these 32 tubes, only in the fimbrial mucosa in 7, and in the mucosa of both the fimbriae and the ampulla in 18. In 7 tubes the fimbriae were not involved but carcinoma was present in the mucosa of the ampulla.

The 7 tubes in which the carcinoma of the mucosa was restricted to that of the fimbriae were carefully studied. Carcinoma was found in the lymphatics of the fimbrial mucosa of only 1 of these tubes and, in this instance, well may have come from the spread of the secondary growth in the fimbrial mucosa and not from the ovarian tumor through the lymph vessels.

In the 18 tubes in which carcinoma was situated in the mucosa of both the fimbriae and ampulla, carcinoma was found in mucosal lymph vessels of both the fimbriae and the ampulla in 16.

In the 7 tubes in which carcinoma was found only in the ampullar mucosa, the fimbriae grossly appeared normal in all, but

only 3 of these fimbriae were examined microscopically. Carcinoma was found in the lymph vessels of the ampullar mucosa in 3 of these 7 tubes.

How may we account for the large number of instances in which the secondary tumors were situated in the distal portion of the tube either in the fimbrial mucosa only or in both the fimbrial and ampullar mucosa? The fimbriated end of the tube is the portion of the tube nearest the ovary and therefore it should be the portion of the tube most likely to be invaded in the continuous extension of the ovarian growth. Since an anastomosis apparently exists between the efferent lymph vessels of the ovary and those of the distal portion of the tube, including the ovarian fimbriae, these vessels offer channels for the spread of the ovarian carcinoma to this portion of the tube. We also must realize that the mucosa of the fimbriae is the portion of the tubal mucosa most exposed to cancer cells which have escaped from the ovarian tumor into the peritoneal cavity. If implantation carcinoma occurs on the tubal mucosa one would expect to find it most frequently in the fimbriae. Since the fimbrial and adjacent ampullar mucosa have a common rich lymph plexus consisting of true capillaries without valves, it is to be expected that if carcinoma reached the vessels of one portion of this plexus by any route it might easily spread to other portions of the same plexus. This might account for the frequent occurrence of carcinoma in the lymph vessels of both the fimbrial and ampullar mucosa of the same tube.

Carcinoma was not found in the lymph vessels of the fimbrial mucosa in 6 of the 7 tubes in which the growth in the tubal mucosa was restricted to that of the fimbriae. If these 6 patients had not been operated upon the carcinoma of the tubal mucosa, later, might have invaded the lymph vessels of the fimbrial mucosa and spread from there to the lymph vessels of the ampullar mucosa and thus presented the picture so often seen in these cases.

Carcinoma of the tubal mucosa secondary to carcinoma of the ovary may arise in the following ways:

1. By the continuous extension of the primary ovarian tumor invading the fimbriae and replacing portions of its mucosa (see Figs. 43 and 50). Carcinoma in the mucosal lymph vessels in some of these cases may come primarily from the portion of the ovarian growth that has replaced the fimbrial mucosa. An extensive ovar-

ian growth may in like manner invade any portion of the tube (see Figs. 19 and 20).

2. By lymphatic metastases or permeation through lymph vessels coming from an ovary in which the primary growth has invaded the lymph vessels of that organ (see Figs. 13 to 17 inclusive).

3. By lymphatic metastasis or permeation arising from the lymph vessels of the mesosalpinx or wall of the tube which has been invaded by an ovarian carcinoma from without.

4. Through the veins (I have been unable to demonstrate this route).

5. By the implantation of cancer cells on the mucosa of the fimbriae and ampulla.

In some instances the determination of the pathogenesis of a given carcinoma of the tubal mucosa is easily made. At other times, especially when the growth is advanced, the diagnosis of its pathogenesis may be very difficult or impossible.

If the secondary tumor presents the histological picture of having arisen from an extensive ovarian growth invading the tubal mucosa, or from the implantation of cancer cells on the tubal mucosa, and if, at the same time, carcinoma is present in the mucosal lymph vessels, it may be impossible to determine whether the carcinoma in these vessels came directly from the tumor in the ovary by metastasis or permeation through the ovarian lymph vessels, or from a downstream spread of the secondary growth in the tube. It might come from both sources in a given case.

Frequently multiple tumors are present in the same tube. It is evident in some instances that the pathogenesis of all of the tumors in a given tube is not the same (see Figs. 43, 46, 47, 90 and 93).

An attempt has been made to group the 32 tubes with carcinoma of their mucosa secondary to carcinoma of the ovary according to the judged pathogenesis of the mucosal carcinoma or to that of the primary or predominating growth when multiple tumors were present.

1. A continuous extension of the ovarian tumor to the fimbriae of the tube replacing a portion of their mucosa was found in seven instances. Carcinoma was observed in the mucosal lymph vessels in all but one of these.

2. Evidence of metastasis or permeation from the ovarian

growth through its lymph vessels as a sole or principal cause of the mucosal carcinoma was found in 6 tubes. In some of the cases in Group "1" as well as those in Group "5" lymphatic metastasis and permeation from the ovarian tumor may have played an important rôle.

3. In 2 tubes the lymphatic origin of the mucosal tumors apparently arose from the invasion of the lymph vessels of the mesosalpinx by carcinoma; in one instance from an extensive ovarian growth which had invaded the mesosalpinx, the isthmus of the tube and the uterine cornu, and in the other case from carcinoma implanted on the mesosalpinx.

4. Implantation of cancer cells on the mucosa was encountered in 16 tubes and was judged to be the sole cause of the secondary mucosal tumors in 9.

5. In 8 tubes the pathogenesis of the predominating mucosal tumor could not be definitely determined. Carcinoma was present in the lymphatics of 6 of these 8 tubes.

Summary of Implantation Carcinoma of the Tubal Mucosa

Implants on the tubal mucosa were found in 12 of the 23 patients with carcinoma of the tubal mucosa secondary to carcinoma of an ovary and, in four instances, occurred in both tubes. They were multiple in 14 of the 16 tubes: frequently various stages in their development could be seen in a single tube. Implants were present on the fimbrial mucosa in 12 of the 16 tubes, in the fimbriae only in 7 and in both the fimbriae and ampulla in 5. In four instances they occurred only in the ampulla. None were found in the isthmus of the tube or on the uterine mucosa.

In 9 tubes implantation was judged to be the sole cause of the secondary carcinoma of the tubal mucosa and also well may have been the cause of the original secondary growth in 3 tubes in which the pathogenesis of this growth could not be positively determined. In 4 instances implants were associated with a secondary carcinoma of the tubal mucosa which obviously had reached the mucosa from the invasion of the fimbriae by an advanced ovarian growth or by lymphatic permeation. A secondary carcinoma of the tubal mucosa due to either of the above phenomena does not prevent the implantation of cancer cells on the mucosa of that tube.

The large number of tubes in which implants were found in the

fimbriae can easily be understood since the fimbrial mucosa is the portion of the tubal mucosa most exposed to cancer cells escaping from the ovarian growth into the peritoneal cavity.

Since cancer cells are frequently found in the lumens of the tubes of patients with carcinoma of the ovary, this portion of their mucosa also may be exposed to these cells. I have observed that they may reach the lumen of the tube in the following ways:

1. From the peritoneal cavity of patients with peritoneal carcinomatosis, through a patent abdominal ostium of the tube (see Figs. 108, 109, 137, 140, 141 and 143).

2. From an ovarian carcinoma in a tubo-ovarian cyst through the patent tubal ostium (see Fig. 23).

3. From the portion of an ovarian carcinoma which has invaded the fimbriae of the tube and then grown through the lumen of the tubal ostium into the lumen of the ampulla (see Figs. 50, 51 and 52).

4. From the invasion of the wall of the tube by an ovarian carcinoma and the subsequent extension of the growth to and through the tubal mucosa (see Fig. 20).

5. From metastatic carcinoma of the tubal mucosa of either lymphatic or implantation origin (see Figs. 92, 26 and 41).

Cancer cells escaping into the lumen of the tube by any route seem to be alive. They apparently may not only live a long time in this situation, but also may increase in numbers, as shown by the presence of mitotic figures in some instances, just as they live and multiply in the lumens of lymph vessels and in the peritoneal cavity. It is to be expected that these cells may become implanted on the tubal mucosa when a suitable soil is created in that mucosa for this phenomenon.

In the present study local reactions were found in both the fimbrial and ampullar mucosa similar to those seen in the serosa of patients with peritoneal carcinomatosis. The form and degree of these reactions varies in individual instances. Sometimes it is slight and consists of a local proliferation of the cells of the subepithelial tissues of the mucosa associated with a partial or a complete loss of the overlying epithelium. In other instances granulation tissue is present on the surface of the mucosa, usually in the form of sessile or pedunculated polypoid outgrowths. Cancer cells are found attached to the areas of the mucosa which show

a local reaction in the subepithelial tissue and a loss of the overlying epithelium. In some instances fibrin is present, holding the cancer cells in place, and at other times fibrin is not evident (see Figs. 37, 111, 114 and 149). Cancer cells are also found attached to or becoming embedded in the outgrowths of granulation tissue just described (see Figs. 28, 30, 65 and 93). In these ways carcinomatous implants arise on the tubal mucosa just as they arise on the peritoneal serosa. As in the latter situation, the mature mucosal implants may be of the following three types: carcinoma replacing portions of the tubal epithelium as though grafted in it (see Figs. 32, 33, 34, 35, 58, 110, 114 and 150), and carcinoma either growing on the surface of or embedded in polypoid newly formed tissue which has arisen on the surface of the tubal mucosa (see Figs. 31, 29, 46, 47, 54, 66 and 70). All stages in the pathogenesis and growth of each of these types of implantation metastases are illustrated and described in this paper. I have not been able to ascertain the exact cause of the local reactions of the peritoneal serosa and of the tubal mucosa which form a fertile soil for the implantation and growth of cancer cells. It is obvious that cancer cells, in some way, are responsible.

Cancer cells in some of the mucosal implants die (see Fig. 47). In other situations, especially in the encapsulated polypoid implants, the carcinoma apparently grows slowly and may be confined to the implant for a long time (see Figs. 66 and 70). It has been shown that the carcinoma in serosal implants may invade the organ or structures beneath them, including the lymph vessels, just as a primary carcinoma invades and spreads. Why should not the carcinoma in mucosal implants do the same?

It has been shown that the granulation tissue on the serosa of one patient with peritoneal carcinomatosis contained lymph vessels, and that the carcinoma in the preexisting lymph vessels of the mesosalpinx from which this granulation tissue had arisen had spread into the newly formed lymph vessels of the granulation tissue (see Figs. 82, 83 and 84). This phenomenon was also observed in granulation tissue which had arisen on the fimbrial mucosa of this patient (see Figs. 94, 95 and 96).

It has been shown that newly formed lymph vessels are present in the stroma of some serosal implants. Circumstantial evidence indicates that carcinoma in these implants may spread through

these newly formed lymph vessels into the preexisting lymph vessels beneath the implant. It is shown in this paper that newly formed lymph vessels are also present in the pedicles of some mucosal implants (see Fig. 71).

One striking anatomical feature of the fimbrial and ampullar mucosa is the richness of its lymphatic plexus, apparently much richer than the lymphatic plexus of the serosa of the ampulla of the tube. It is apparently more superficial and, therefore, more accessible to injury and stimulation than the latter. When one encounters a secondary carcinoma of the tubal mucosa with the histological structure of an implant and carcinoma in the lymph vessels of the mucosa beneath this tumor with evidence that the carcinoma in the lymph vessels is either continuous with or closely related to the secondary mucosal carcinoma, one is tempted to believe that this secondary carcinoma is an implant and that cancer cells from it may have spread into the underlying lymph vessels (see Figs. 107, 111 and 138). The cancer cells from an implant might, in this way, spread to the lymph vessels of the mesosalpinx and even reach the ovary, the site of the primary carcinoma from which the original cancer cells of the implant came.

Since carcinoma in the preexisting lymph vessels of the mesosalpinx and tube sometimes spreads into the lumens of newly formed lymph vessels in granulation tissue arising on both the serosa and the tubal mucosa (see Figs. 82, 83, 84, 94, 95 and 96), it might subsequently invade the granulation tissue about these vessels. A condition would thus arise simulating the extension of the carcinoma in an implant into the newly formed lymph vessels of its stroma. I have never recognized this phenomenon in granulation tissue on the tubal mucosa, but have seen it in similar tissue on the serosa. A still more confusing picture would arise if cancer cells became embedded in the granulation tissue just described before or after the spread of carcinoma in preexisting lymph vessels into the newly formed lymph vessels in the granulation tissue.

A differential diagnosis between mucosal implantation metastases and metastases to the tubal mucosa through the lymph vessels usually may be made easily in the early stages of these secondary tumors. However, in the advanced stages of either of these metastases a positive diagnosis of their pathogenesis may be very difficult or impossible.

Conclusions

The following observations were made during the study of 16 tubes in which judged carcinomatous implants on the mucosa, secondary to carcinoma of the ovary, were found.

1. Changes in the tubal mucosa favorable for the implantation of cancer cells on its surface, such as: (*a*) local proliferations of the cells of the subepithelial tissues of the mucosa associated with a partial or a complete loss of the overlying epithelium; and (*b*) granulation tissue of various types on the surface of the mucosa, usually in the form of sessile or pedunculated polypoid outgrowths in some of which newly formed lymph vessels were detected.

2. Cancer cells splinted by fibrin on areas of the mucosa with a local reaction of their subepithelial tissues and also cancer cells attached to these areas without the presence of fibrin. Cancer cells attached to or becoming embedded in the polypoid granulation tissue just described.

3. Carcinoma replacing portions of the tubal epithelium as though grafted in it. Other neoplasms consisting of carcinoma either growing on or embedded in organized newly formed tissue attached to the surface of the mucosa, usually in the form of sessile or pedunculated polypoid tumors.

4. All stages in the development of each of these three types of mucosal implantation metastases.

5. Sometimes implanted carcinoma dies. In other instances it grows slowly and apparently remains localized for a long time. Again conditions are found indicating that implanted carcinoma has invaded the mucosal tissues beneath it including the lymph vessels just as a primary carcinoma invades and spreads.

6. The pathogenesis, structure, form and life history of carcinomatous implants of ovarian origin on the tubal mucosa are the same as the pathogenesis, structure, form and life history of similar implants on the peritoneal serosa.

Note: The efficiency of the laboratory work for this paper is, in large measure, due to the technical skill and the interest of Miss Helen Buchan. The illustrations of the gross specimens were made by Mrs. M. R. Marden and the microphotographs by Mr. James A. Glenn. These I thank for their interest and cooperation.

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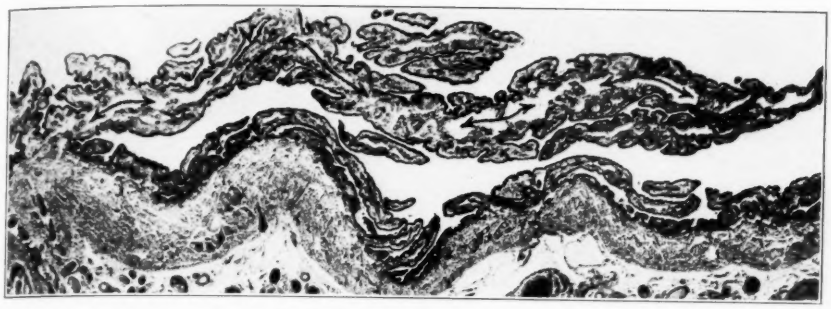
DESCRIPTION OF PLATES

PLATE 78

FIG. 1. Microphotograph of a longitudinal section of the distal portion of the ampulla of a normal appearing tube. The patient, A. H. No. 26207, nulliparous and aged 43 years, had had the appendix, the entire uterus and both tubes and ovaries removed for carcinoma of the body of the uterus. The hysterectomy had been preceded by the intrauterine application of radium followed by deep X-rays. The tube had been incised longitudinally before placing it in formalin in order to avoid the compression of its mucosa incident to the fixation of an intact tube. A large mucosal fold and many smaller folds, beneath it, are shown in this microphotograph. This section includes only a small portion of the base of the large fold at the left. Note the large lymph vessel running longitudinally in the large fold. Since it is a true capillary without valves, cancer cells gaining access to its lumen may easily spread in all directions in the lymph plexus of the fold, even into the fimbrial mucosal fold which is a continuation of this ampullar fold. Note also a similar arrangement of the lymphatics in the smaller folds. A reading glass is of great value in studying all of the microphotographs. × 10.

FIG. 2. The pattern of the lymphatics of the mucosa of the ampulla of a normal appearing tube as seen in cross section. The patient, A. H. No. 28, parous and aged 40 years, had had the appendix, the entire uterus and one tube and ovary removed for an adherent retroflexed uterus associated with peritoneal endometriosis. The tube had been incised longitudinally before placing it in formalin. Irrespective of the size of the mucosal folds the general pattern of their lymphatics is the same. The lymphatics in the folds empty into vessels at the base of the folds which are continuous with vessels between the folds. Thus the lymphatics of one mucosal fold are united with those of adjacent folds. The lymphatics of the secondary mucosal folds empty at their base into the vessels of the primary folds just as the lymph vessels of the primary folds drain into the vessels at their base. Note that while the lymphatics tend to be centrally situated in the folds they may be, in places, close to the epithelium. A portion of a large mucosal fold appears at the right. A longitudinal section through such a fold might not include its base, as occurred in the preceding section. × 25.

FIG. 3. Microphotograph similar to the preceding one. The patient, A. H. No. 699, parous and aged 40 years, had had the appendix, the entire uterus and one tube and ovary removed for a uterine leiomyoma. The pattern of the lymphatics of the mucosa of the ampulla, as seen in cross section, is the same as that shown in the preceding microphotograph. A study of the lymphatics of the mucosa of the ampulla of many tubes has convinced me that in a general way this pattern is constant with minor variations according to the size and complexity of each mucosal fold. × 25.



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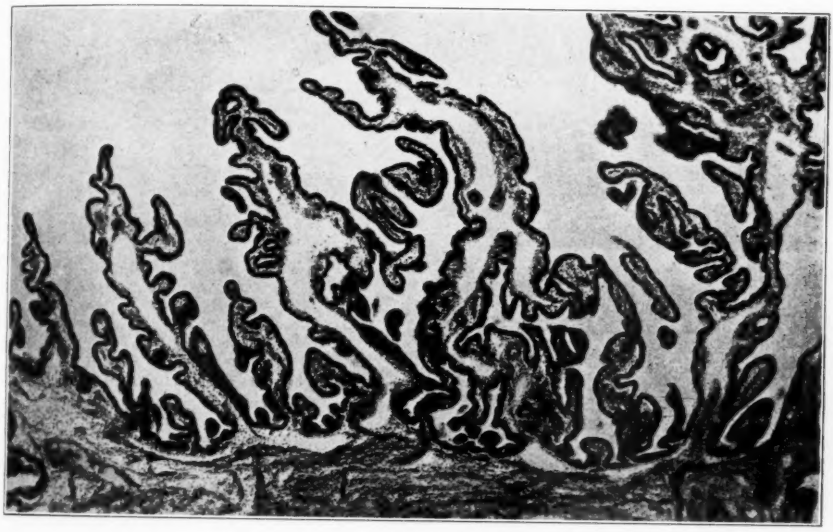

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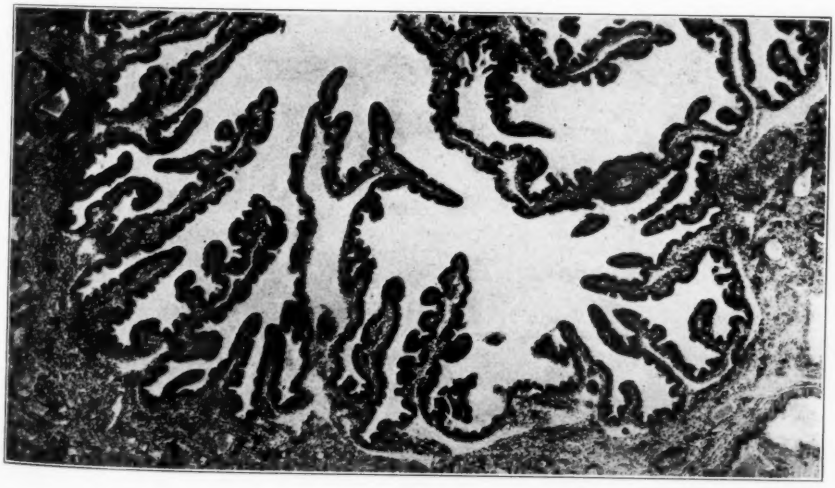

3

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Implantation Carcinoma of Tubal Mucosa

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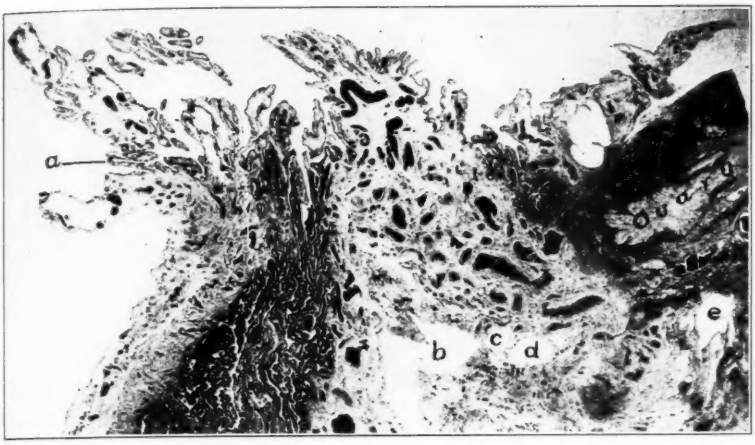
FIG. 4. Longitudinal section of the distal portion of a normal appearing tube including its fimbriae proper, the mesosalpinx with its ovarian fimbriae, and the tubal pole of the ovary at the right. The patient, A. H. No. 9339–31, parous and aged 47 years, had had the entire uterus and one tube and ovary removed for a uterine leiomyoma. After removing the uterus and before releasing the clamp on the ovarian vessels these vessels were ligated. A ligature was also placed about the tube, broad ligament and utero-ovarian ligament, near the uterus. The tube and ovary were then severed from the uterus and immediately placed in formalin. The veins thus filled with blood, as with an injection mass, appear black in the microphotograph. Their distribution suggests an intimate relationship between venous outlets of the tubal pole of the ovary and those of the fimbriae and the mucosa of the distal portion of the tube. The large irregular space "b" arose from an incomplete section of the mesosalpinx. I believe that the spaces "c," "d" and "e" are the lumens of large lymph vessels. Under higher magnification occasional constricted lymph vessels can be seen in the compressed folds of the mucosa of the ampulla and dilated lymph vessels, in many of the folds of the fimbrial mucosa. The fimbrial mucosa is but a continuation of the mucosa of the ampulla beyond the ostium of the tube. The lymphatics in the mucosa in the two situations are continuous (see Fig. 9 of previous paper[11]). Carcinoma gaining access to the lymphatics of the fimbrial mucosa could easily spread to those of the ampullar mucosa and *vice versa*. This might account for the occurrence of carcinoma in both situations in many instances. × 5.

FIG. 5. Higher magnification of a portion of the fimbrial mucosa to the right of the abdominal ostium of the tube shown in the preceding microphotograph. The pattern of the lymphatics in the mucosal folds of the fimbriae and ampulla shown in Figures 2 and 3 is the same. × 25.

FIG. 6. Higher magnification of a portion of the mucosal folds of the fimbriae indicated by "a" in Figure 4. Note that while the lymph vessels in the mucosal folds are for the most part more centrally located than the blood vessels, in places they are separated from the peritoneal cavity by only a very thin layer of tissue covered by epithelium. This proximity of the lymph vessels to the surface of the mucosa suggests not only their accessibility to carcinoma of the tubal mucosa, but also their availability for the origin of newly formed lymph vessels as well as newly formed blood vessels in granulation tissue which, in some instances, arises on the surface of the mucosa (see Figs. 71, 95 and 96). × 54.



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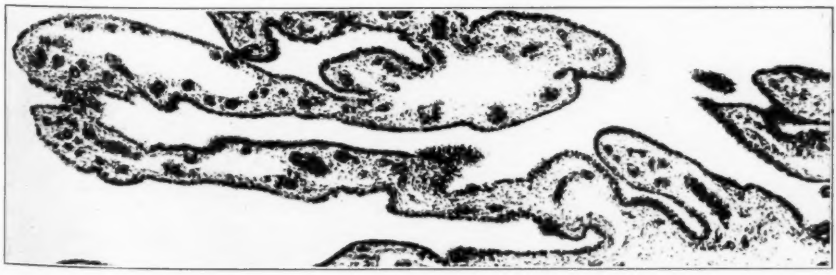

6

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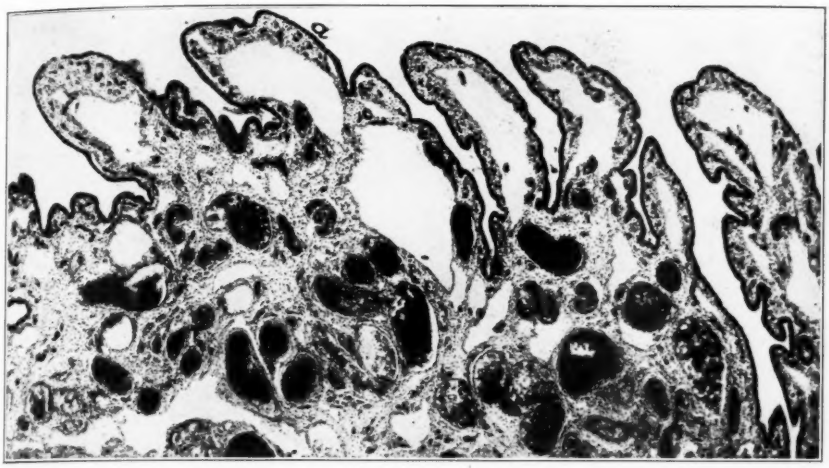
PLATE 80

FIG. 7. Longitudinal section of a portion of the fimbrial mucosa of a normal appearing tube. The patient, A. H. No. 28737, parous and aged 42 years, had had the entire uterus and one tube and ovary removed for a uterine leiomyoma. The veins partially or completely filled with blood are evident. The dilated lymphatics, by contrast, are just as striking as the veins. Note the central location of the large lymph vessels in the mucosal folds and the situation of the blood vessels between them and the surface of the folds. × 25.

FIG. 8. Higher magnification of a portion of the mucosal fold "a" of the preceding microphotograph. The central location of the large dilated lymphatic in this fold with the blood vessels situated between it and the surface of the mucosa is evident. However, note that a portion of the mucosal lymph plexus "a" and "b" is situated between these blood vessels and the surface of the fold and is separated from the epithelium by only a very thin layer of tissue. The close proximity of portions of the lymph plexus of the ampullar and fimbrial mucosa to the surface of the mucosa is of great importance in the present study. Carcinoma growing in the lymphatics of the mucosa may easily push through this thin layer of tissue and overlying epithelium into the lumen of the tube or into the peritoneal cavity, as will be shown. Carcinoma replacing the overlying epithelium in such an area may easily invade the nearby lymphatics. One of these superficial lymph vessels might take part in the formation of newly formed lymph vessels in granulation tissue arising on the surface of such an area of the mucosa (see Figs. 95 and 96). × 130.

FIG. 9. Longitudinal section of a portion of the fimbriae of a normal appearing tube at a mucoserosal junction ("a"). The patient, A. H. No. 1698–32, parous and aged 45, had had the entire uterus and left tube and ovary removed and the pelvic floor repaired for descensus of the uterus associated with a follicular cyst of the ovary and a relaxed pelvic floor. The veins partially or completely filled with blood are evident. All of the empty spaces are lymphatics except "b," which is a small mesothelial cyst. The dilated lymphatics of the mucosa apparently drain into deeper lymph vessels of the tubal wall. Lymph vessels are not evident in the serosa at the right. The richness of the mucosal lymphatics and their fitness for the spread of carcinoma in them in this situation are evident. Their proximity to the surface of the mucosa exposes them to any pathological changes affecting the surface of the fimbriae. × 25.



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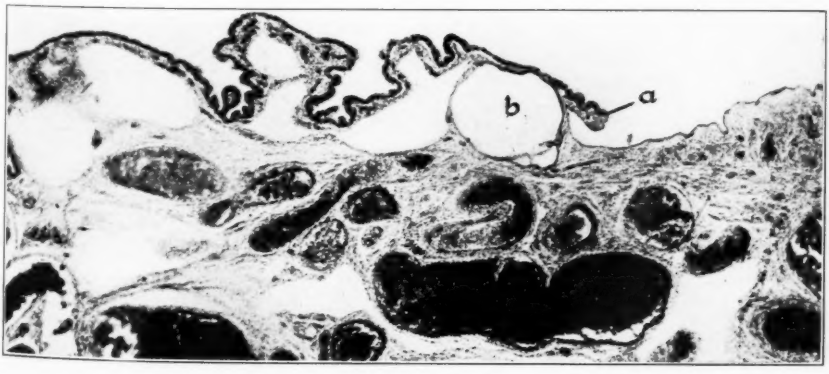

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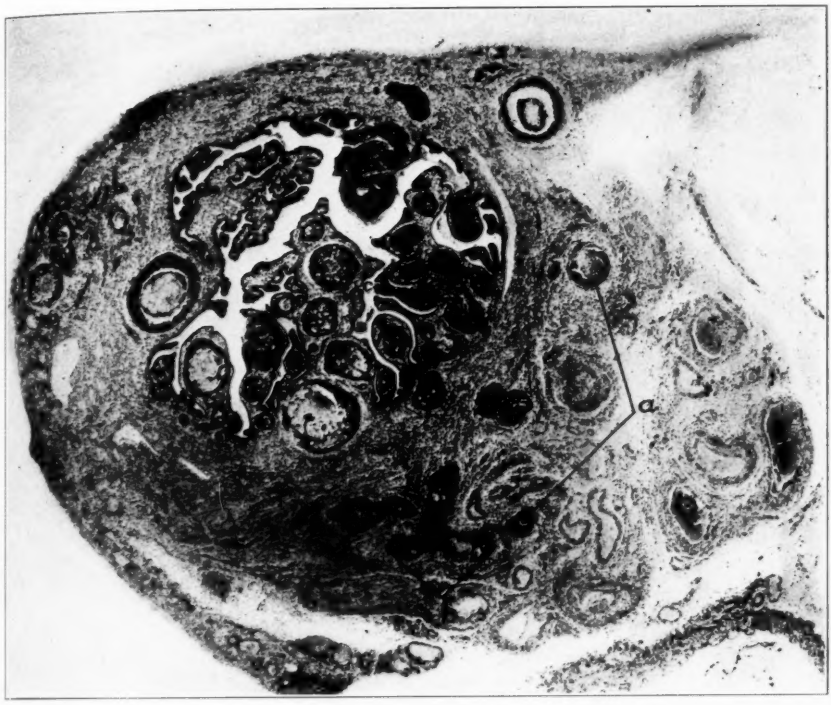
Plate 81

Fig. 10. Cross section of the ampulla of a tube from a patient, A. H. No. 4727–31, with carcinoma of the tubal mucosa and of the ovary secondary to that of the uterus as a result of lymphatic permeation from the uterine tumor (see also Figs. 18 and 19, and Figs. 5, 6 and 7 of two previous papers, [1,11]). Lymph vessels "a" of the mesosalpinx are filled with carcinoma. A collecting lymph vessel extending from the tube into the mesosalpinx, indicated by "b," demonstrates a channel by which the carcinoma in the lymph vessels of the mesosalpinx may have reached the mucosal lymphatics. The mucosal lymphatics are greatly distended with the carcinoma which in one situation ("c") has grown through the wall of the mucosal lymph vessel into the lumen of the tube. Note the scarcity of the subserosal lymphatics as compared with those of the mucosa if their injection with carcinoma is complete. Since carcinoma of the tubal mucosa and of the ovary may arise from carcinoma of the uterus by extension through the lymph vessels we would expect that carcinoma of the tubal mucosa might arise from the ovary through similar channels. × 16.

Fig. 11. A portion of a cross section of the ampulla of the left tube in which the mucosal lymphatics are filled, as with an injection mass, with carcinoma secondary to that of the left ovary (Case 1). The distribution of the carcinoma in the mucosa is similar to that of the carcinoma in the preceding microphotograph. The pattern of the carcinomas in the mucosa is the same as that of the lymph vessels shown in Figures 2 and 3. Since the lymph vessels of the ampullar mucosa, shown in the preceding microphotograph, were injected with carcinoma by lymphatic permeation from a primary growth in the uterus it is conceivable that the carcinoma in the present section may have reached its present situation by a similar route from the ovary. × 54.



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11

Sampson Implantation Carcinoma of Tubal Mucosa

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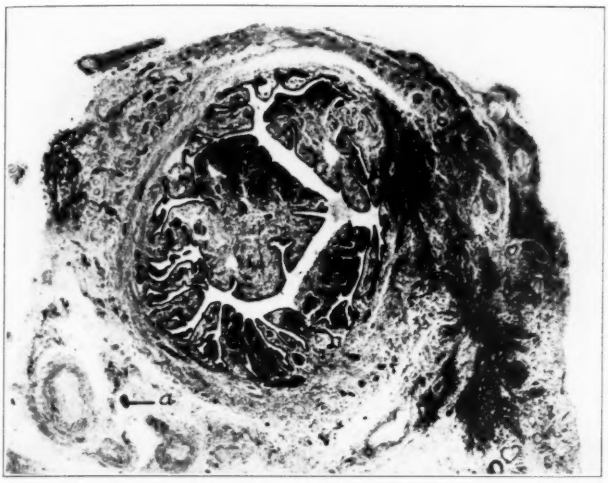
PLATE 82

FIG. 12. Cross section of the entire tube, a portion of which is shown in the preceding microphotograph (lower magnification). Carcinoma is present in lymph vessels in all layers of the tube including the mesosalpinx "a," as in the tube shown in Figure 10. In places the growth has escaped from the lymph vessels and infiltrated the tubal tissues like the extravasation of an injection mass through a ruptured lymphatic. × 16.

FIG. 13. Section of a small portion of the ovarian carcinoma of Case 1, the mesosalpinx and ovarian fimbriae. The fimbriae (above) are greatly thickened and distorted by carcinoma which has infiltrated their tissues. Carcinoma is also present in lymph vessels which accompany the blood vessels. The latter are very distinct, even with this low magnification. The ovarian carcinoma has invaded the tissues of the mesosalpinx and is barely visible in small lymph vessels (see arrow), apparently making its way toward the fimbriae above. × 10.

FIG. 14. Higher magnification of the field indicated by the arrow in the preceding microphotograph. (This print is mounted at right angles to the preceding one.) Above and below the arrow, carcinoma is shown in lymph vessels apparently extending from the ovarian carcinoma into the mesosalpinx. Carcinoma also is present in a lymph vessel ("a"). The condition found in the last two microphotographs indicates that the carcinomas in the lymphatics of the tubal mucosa shown in Figures 11 and 12 could have reached their present situation, through the lymph vessels, from the ovarian carcinoma. × 54.



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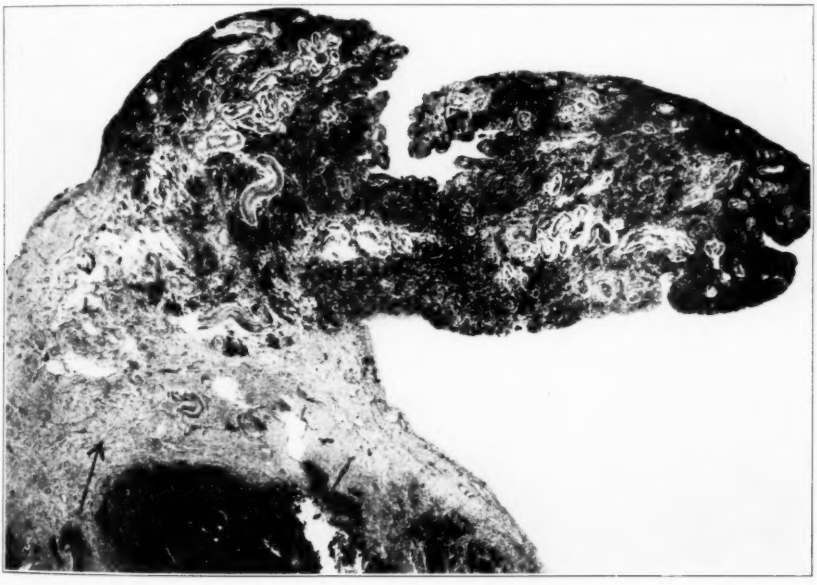

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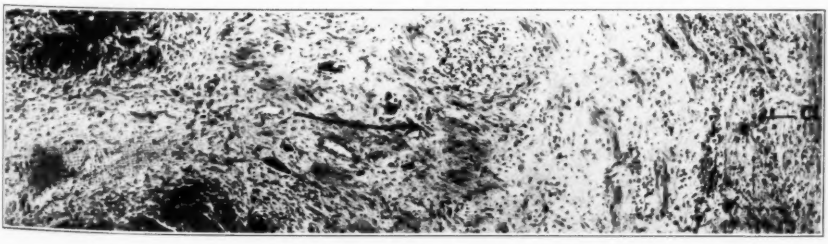

14

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Implantation Carcinoma of Tubal Mucosa

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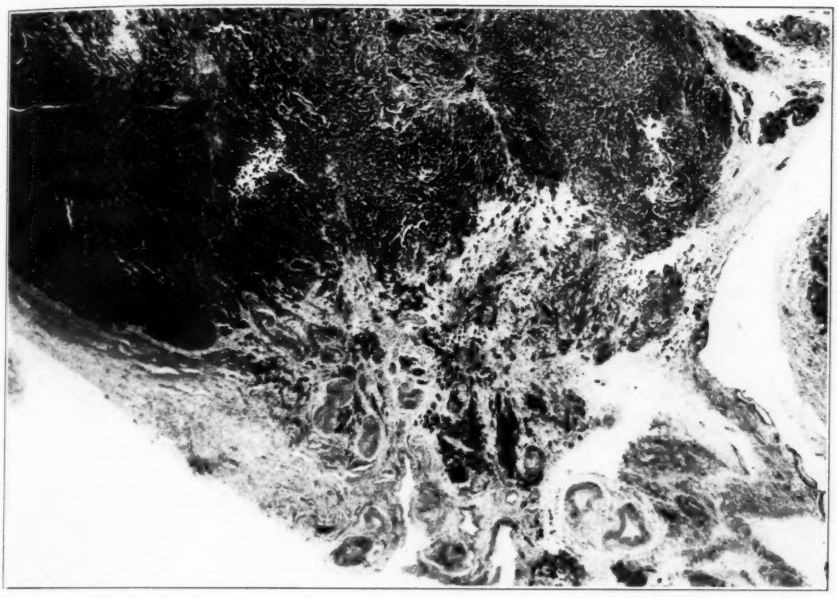
PLATE 83

FIG. 15. Section of a portion of the left ovary including its hilum (Case 2). It shows well the extension of the carcinoma beyond the ovary into the tissues of its hilum in lymph vessels which accompany the blood vessels. If secondary carcinoma is present in the mucosa of the accompanying tube, as the result of lymphatic permeation or metastasis, we would expect to find it primarily in the lymph vessels. × 10.

FIG. 16. Longitudinal section of the distal portion of the left tube including its fimbriae (Case 2). The tissues of the fimbriae at the right and toward the ovary are infiltrated with carcinoma as in Figure 13. Carcinoma is present in many of the lymph vessels of the wall of the tube. The carcinoma to the left of the large arrow may well mark a lymphatic which normally drains the mucosal lymph vessels, and in this instance indicates a channel through which the carcinoma of the ovary may have reached the mucosa of the fimbriae. A mucosal fold of the ampulla at the left is also infiltrated with carcinoma (× 10). The small arrow and the higher magnification (× 130) of this field in the insert above demonstrate what I believe to be the outgrowth and extension of the carcinoma of the deeper tissues of the mucosa into the lumen of the tube and not the implantation of cancer cells on the surface of the mucosa and their subsequent invasion of the tissues of the mucosa.



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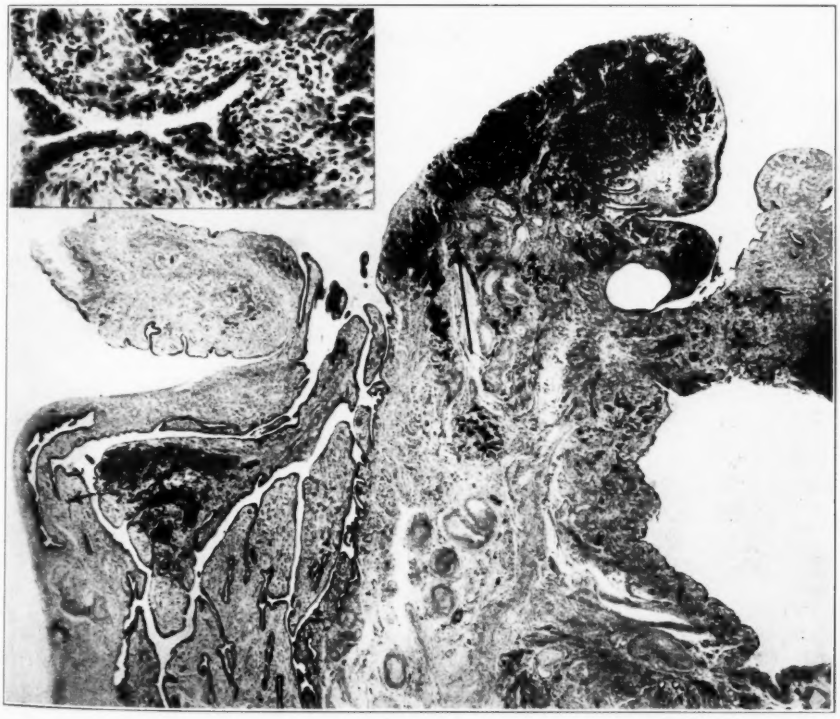

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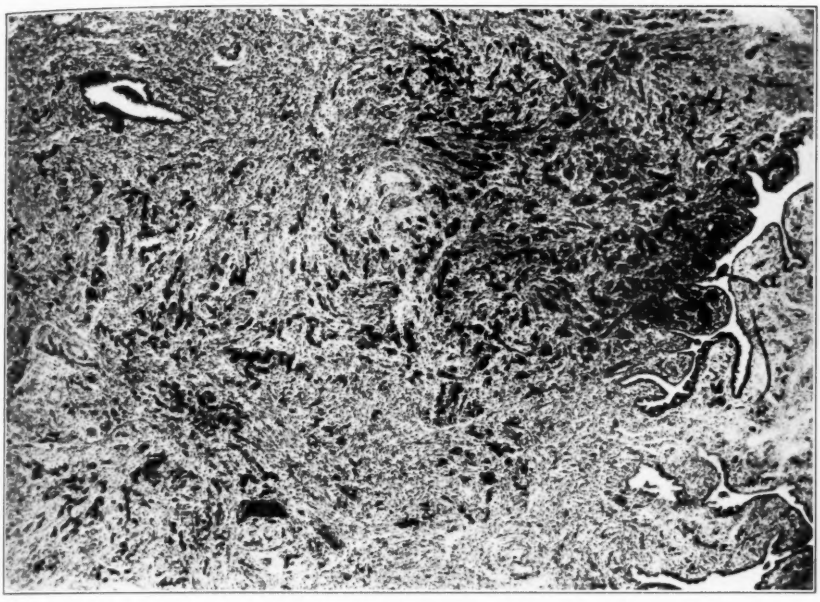
Implantation Carcinoma of Tubal Mucosa

PLATE 84

FIG. 17. Longitudinal section of a portion of the proximal end of the ampulla of the tube shown in the preceding microphotograph. The wall of the tube is infiltrated by carcinoma which I believe is situated, for the most part, in lymphatics. The mucosa, at the right, is likewise infiltrated with carcinoma. Carcinoma "a" is present in the lumen of the tube and probably reached its present situation by the extension of the tumor in a mucosal lymph vessel through the overlying epithelium and is not a clump of cancer cells which escaped into the peritoneal cavity from the ovarian tumor and was subsequently swept into the lumen of the tube. All of the evidence found in the study of the material from this case points to the origin of the carcinoma of both the fimbrial and the ampullar mucosa by lymphatic permeation and metastasis from the ovarian tumor and not from the implantation of cancer cells on the tubal mucosa. Since ovarian carcinoma can reach the tubal mucosa through the lymph vessels, tubal carcinoma, both primary and secondary, might be able to reach the ovary by the same route. × 25.

FIG. 18. Carcinoma of the proximal portion of the ampulla of the left tube secondary to carcinoma of the ovary (see the next microphotograph (Case 3)). The carcinoma in places ("a") has not only extended to the epithelium of the mucosa but has replaced it, as in Figure 16 (compare with the preceding microphotograph). I believe that the pathogenesis of the carcinoma of the ampullar mucosa in the two sections is the same. × 25.



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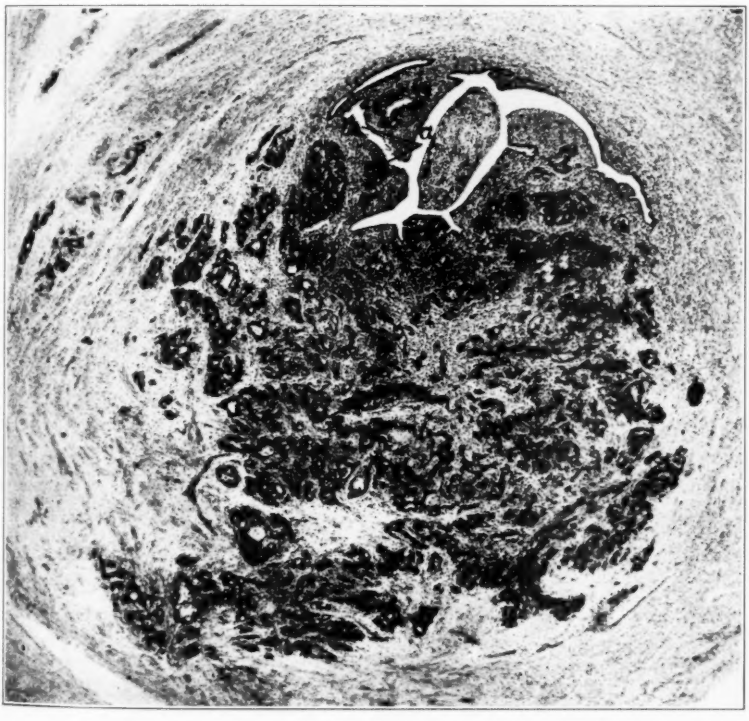

18

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PLATE 85

FIG. 19. Coronal section of the left uterine cornu and the proximal portion of the tube including a portion of the ovarian carcinoma which has invaded both the uterus and the tube (Case 3). The ovarian carcinoma is partly necrotic and here it has failed to take the stain. The invasion of the uterine cornu, the mesosalpinx and walls of the isthmus of the tube by the growth is well shown. Carcinoma ("c") is also present in a lymph vessel of the mesosalpinx. Portions of the isthmus of the tube ("a and b") are necrotic and in both situations are surrounded by tissue infiltrated by the growth. The section shown in the preceding microphotograph of this case is from the same series of sections as this one. × 5.

FIG. 20. Carcinoma of the mucosa of the uterine portion of the tube from the same series of sections as the one shown in Figure 18. The pathogenesis of the mucosal carcinoma in the two situations is the same. × 54.

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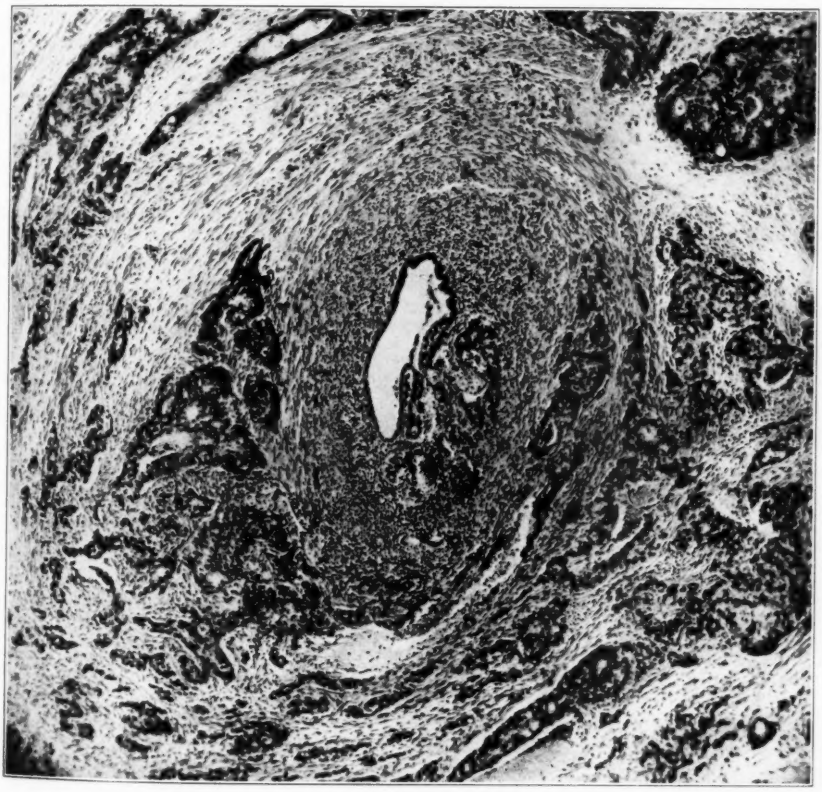

20

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PLATE 86

FIG. 21. Cross section of the left tube and ovary from a patient with carcinoma of the left ovary associated with peritoneal carcinomatosis (Case 4). Carcinoma is present in the ovarian fimbriae adherent to the ovary and grossly appears to have arisen by direct extension from the growth in the ovary. Natural size.

FIG. 22. Longitudinal section of the tubal fimbriae shown in the preceding illustration through the carcinoma involving the ovarian fimbriae. The histological structure of the neoplasm in this situation is that of an advanced growth which has invaded the fimbriae from within and spread in their tissues as from lymphatic permeation (compare with Figs. 13 and 16). Carcinoma is present in lymph vessels in the tissues adjacent to the left hand portion of the tumor. An occasional small embolus of cancer cells can be found in lymph vessels in all parts of the tubal wall proximal to the fimbriae. The continuity of the fimbrial carcinoma with the primary ovarian growth, so clearly portrayed in the previous illustration that it must have been present, was not seen in the incomplete microscopic study of this area. × 10.

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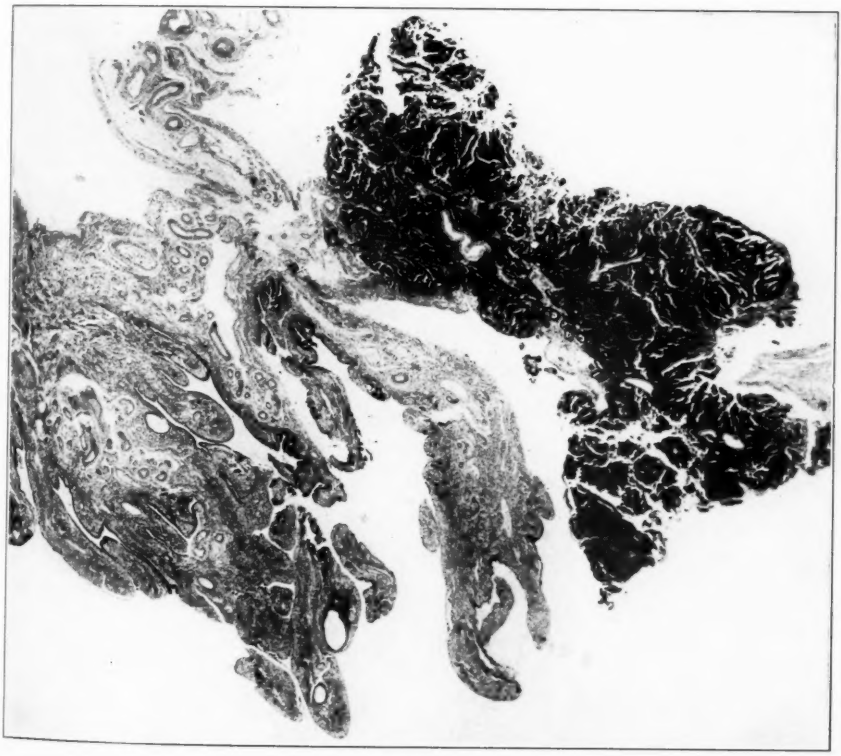

22

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Implantation Carcinoma of Tubal Mucosa

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PLATE 87

FIG. 23. Coronal section through a tubo-ovarian cyst showing multiple carcinomas of various sizes of the lining of the ovarian cyst, and also multiple, very small carcinomas of the mucosa of the ampulla of the tube (Case 5). The multiple tumors in both the ovary and the tube indicate that they are of either multicentric origin or one parent growth with the other tumors secondary. The great variation in the size of the tumors, most evident in the ovarian cyst, suggests a variation in their ages. This supports the theory of a primary growth with metastases, but does not exclude the possibility of the same carcinogenetic factors activating the epithelial lining of both the ovarian cyst and the tube at different times. The epithelial lining of the ovarian cyst is continuous with and histologically indistinguishable from the epithelium of the tube. The tumors are all superficial. Carcinoma was not found in the lymph vessels of either the ovary or the tube. If a primary growth is present it obviously should be the largest one in the ovary. Natural size.

FIG. 24. One of the small discrete tumors of the ovarian cyst. It is a typical papillary carcinoma which, in this situation, has spread over the lining of the cyst by replacing the epithelium of the latter. Tumor cells may readily escape from such a growth into the cavity of the ovarian cyst as well as from the largest and judged parent tumor shown in the preceding illustration. Similar cells would also migrate into the lumen of the patent tube (see arrow in Figure 23). × 5.

FIG. 25. Section of the tubo-ovarian cyst at the tubo-ovarian junction, corresponding to the field bounded by the dotted lines and the cavity of the tubo-ovarian cyst, marked "a" in Figure 23. Multiple small papillary tumors of various sizes are shown on the lining of the ovarian cyst at the left which in this situation consists, at least in part, of tubal fimbriae fused with the wall of the cyst (see pointer "a"). Similar papillary tumors are present also on the tubal mucosa, above and at the right. × 8.

FIG. 26. A portion of the ampulla of the tube, from a block adjacent to the preceding one, demonstrating the further spread of the tumors on the mucosa of the tube. × 10.

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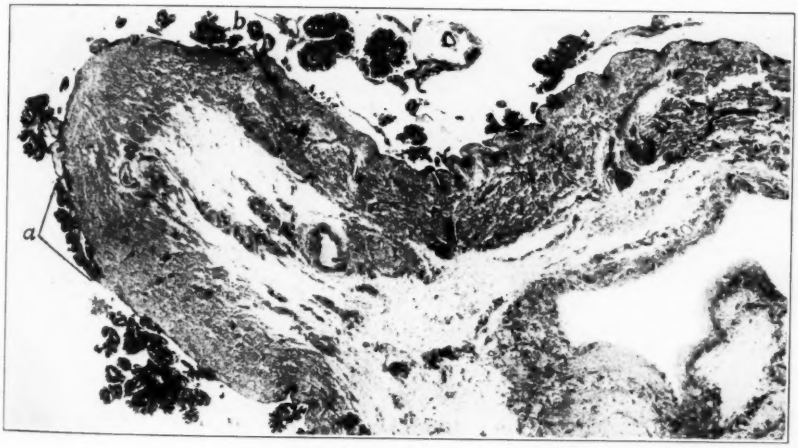

25

26

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Implantation Carcinoma of Tubal Mucosa

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FIG. 27. Granulation tissue which has arisen on the surface of the tubo-ovarian cyst through breaks in its epithelial lining. This tissue arose from the portion of the ovarian cyst lined by tubal fimbriae. The distal portion of this outgrowth is shown, in the series of sections, to be attached to the tip of a judged mucosal fold of the fimbriae. Note the sessile round polypoid mass of newly formed tissue marked "a" and the pedunculated mass of younger granulation tissue above it. Outgrowths of tissues, like these, are frequently seen on the serosa of patients with peritoneal carcinomatosis. Cancer cells floating about in the peritoneal cavity become attached to and embedded in these outgrowths. Since this tissue forms the stroma of one type of peritoneal implant it might also form the stroma of a similar type of mucosal implant. × 54.

FIG. 28. The distal and youngest portion of the newly formed tissue shown in the preceding microphotograph from another section in this series. A clump of cancer cells is embedded in this tissue which consists of fibrin and cells derived from the subepithelial tissues of the fimbrial mucosa. The majority of these migratory cells are judged to be fibroblasts. A few polymorphonuclear leukocytes and lymphocytes are present also. Newly formed blood vessels have not yet appeared in the tissue shown here. Phenomena like this are frequently encountered in patients with peritoneal carcinomatosis and present early stages in the pathogenesis of one type of carcinomatous implant. × 130.

FIG. 29. A pedunculated polypoid carcinomatous implant on the mucosa of the ampulla of the tube, shown in Figure 23, near the isthmus. Clumps of cancer cells are embedded in the body of this implant. Its slender vascular pedicles are similar to those of the polypoid outgrowth of granulation tissue shown in Figure 27. The pathogenesis of this implant is suggested in the two preceding microphotographs. Polypoid implants, like this one, are frequently encountered on the serosa of patients with peritoneal carcinomatosis where all stages in their development have been studied.[9] × 54.

FIG. 30. The body of a pedunculated polypoid outgrowth of newly formed tissue (see "a" of Fig. 26), which has arisen from the mucosa of the ampulla of the tube. The histological structure of this tissue is similar to but older than the sessile, newly formed tissue marked "a" in Figure 27. Clumps of cancer cells are splinted by fibrin on the surface of this tissue, at the right. The condition shown here represents on early stage in the possible implantation of cancer cells on newly formed tissue derived from the tubal mucosa (see the next microphotograph). The pedicle which unites this tissue to the tubal mucosa beneath it doesn't appear in this section. The black area marked "a" may be the remains of cancer cells which became embedded in this tissue but failed to survive (see Fig. 39). × 130.

FIG. 31. The body of a pedunculated polypoid outgrowth of newly formed tissue similar to the tissue shown in the preceding microphotograph and, like the latter, arising from the mucosa of the ampulla of the tube. Carcinoma is growing on the surface of this tissue which is apparently younger than the tissue shown in Figure 30. I believe that the condition shown here represents a stage in the development of one type of polypoid implant later than that illustrated in the preceding microphotograph. The slender vascular pedicle of this implant appears in Figure 40. × 130.

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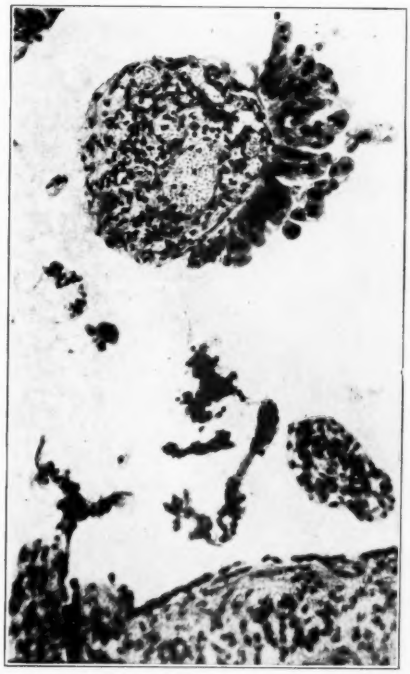

31

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Implantation Carcinoma of Tubal Mucosa

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FIG. 32. Carcinoma ("a") growing on the surface of the tip of a mucosal fold of the fimbriae in the field marked "a" shown in Figure 25 but from another section in this series. Note that the epithelium covering this portion of the fold has almost entirely disappeared, thus creating a soil suitable for the implantation and growth of cancer cells. Even some of the fibrin ("b" and "c") which may at one time have held the cancer cells against the tubal mucosa, remains (see Fig. 30). The condition shown here represents an early stage in the growth of cancer cells implanted on the tubal mucosa without the assistance of polypoid granulation tissue as observed in Figures 30 and 31. × 130.

FIG. 33. A stage in the growth of carcinoma implanted on the tip of a mucosal fold of the tube later than that shown in the preceding microphotograph, from the same series of sections as the section shown in Figure 26. Fibrin ("a") which at one time may have splinted the cancer cells on the tubal mucosa still remains. × 130.

FIG. 34. A still later stage in the growth of carcinoma implanted on the tip of a mucosal fold of the ampulla, from the same series of sections as the preceding one. The papillary structure of this growth is more evident than the one shown in Figure 33. However, the implant is still immature (see the mature implants shown in Figs. 35 and 41). Note a sharp demarcation between the carcinoma and the tubal epithelium and that the carcinoma does not present the appearance of having arisen from that epithelium. × 130.

FIG. 35. Higher magnification of the ampullar mucosa with carcinoma on its surface marked by pointer "b" in Figure 26. The carcinoma, at the left, is splinted against the mucosa by fibrin. For a higher magnification of this field see the next microphotograph. One may judge that the reaction of the subepithelial tissue of the mucosa is caused by the carcinoma above it. This carcinoma can be shown in the series of sections to be implanted already on the tip of a slender mucosal fold, similar to the carcinoma in Figure 32. Later it might have become implanted on the mucosa, shown here, if the patient had not been operated upon. The carcinoma at the center and right represents a mature implantation of cancer cells on the tubal mucosa. × 105.

FIG. 36. Higher magnification of the left hand portion of the field shown in the preceding microphotograph. The fibrin, splinting the carcinoma, has been invaded by cells derived from the subepithelial tissues of the mucosa beneath the carcinoma. The majority of these migrating cells are judged to be fibroblasts. A few polymorphonuclear leukocytes and lymphocytes are present. These cells are passing through and between the epithelial cells which may be injured by the nearby carcinoma, thus permitting the escape of fibrin and migratory cells into the lumen of the tube. × 170.

FIG. 37. Higher magnification of the central portion of the tubal mucosa shown in the next microphotograph. Clumps of embolic cancer cells are splinted on this mucosa by fibrin. At the left, the tubal epithelium has disappeared. A clump of cancer cells ("a") is in contact with the resulting raw area, but may not be actually implanted in this situation. However, to the right of the center another break in the tubal epithelium occurs (see pointer "b") with carcinoma definitely implanted on this area of the mucosa. A later stage in the phenomena shown here may result in the condition pictured above in Figure 35. × 130.

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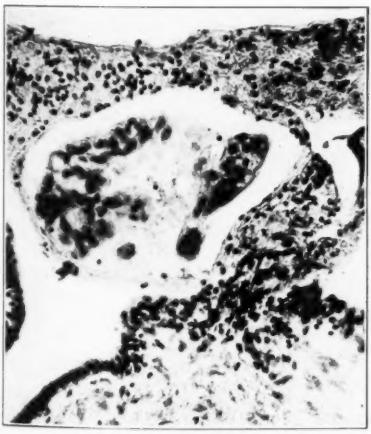

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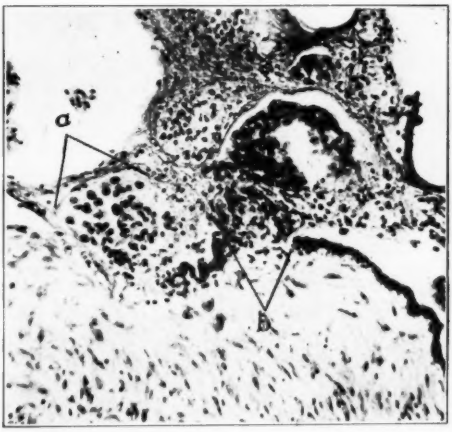

37

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Implantation Carcinoma of Tubal Mucosa

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PLATE 90

FIG. 38. Carcinoma of the tubal mucosa from the same section shown in the preceding microphotograph. Note the tall polypoid growth with a long slender pedicle. This pedicle is covered with tubal epithelium. I believe that this tumor is a mature implant either on the tip of a slender mucosal fold or on polypoid, pedunculated, newly formed tissue (see Fig. 40). If on the latter, the tubal epithelium has grown over the surface of the pedicle of the implant. The judged early implantation of embolic clumps of cancer cells, shown in Figure 37, appears in the center of this field. A portion of a mature implant, similar to the one shown in Figure 35, appears at the right. × 54.

FIG. 39. Cancer cells, singly and in clumps, enmeshed in fibrin with evidence of the death of two of the cancer cells in the largest clump. The cancer cells in the clump marked "a" also appear to be dead. Compare with the judged living cancer cells below them. In the majority of instances in this specimen the cancer cells on the surface of or enmeshed in fibrin stained as well as the tubal epithelium and were judged to be alive when the patient was operated upon. Apparently they may live a long time in the lumen of the tube without implantation on vascular tissue. Evidence of mitosis was not observed in any of these cells nor was it detected in the many carcinomas of the tubal mucosa which were carefully examined for this phenomenon. × 170.

FIG. 40. The early implantation of cancer cells on polypoid granulation tissue arising from the tubal mucosa. This is the same early implant shown in Figure 31, from another section in this series. The greater portion of its long, slender vascular pedicle appears in this section (compare also with Figures 27 and 29). Had the epithelium of the tube grown over the pedicle of this implant it later might have presented a picture similar to that shown above in Figure 38. Clumps of judged dead cancer cells are marked "a." × 107.

FIG. 41. Higher magnification of the mature implant marked "b" in Figure 25. It evidently arose from the growth of cancer cells implanted either on the tip of a mucosal fold, as shown in Figures 32, 33 and 34, or on the surface of polypoid, newly formed tissue, similar to that pictured in the preceding microphotograph. Clumps of viable cancer cells might easily escape into the lumen of the tube from the tips of the growing papillary processes of this and other similar neoplasms shown in this tubo-ovarian cyst. × 107.

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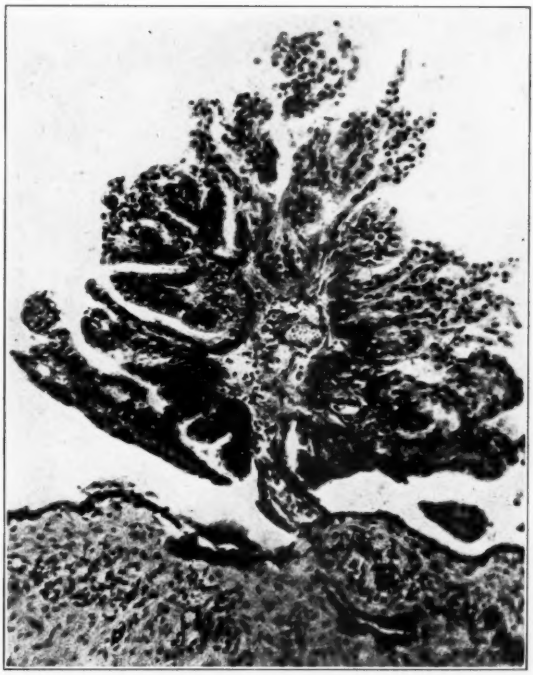

41

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Implantation Carcinoma of Tubal Mucosa

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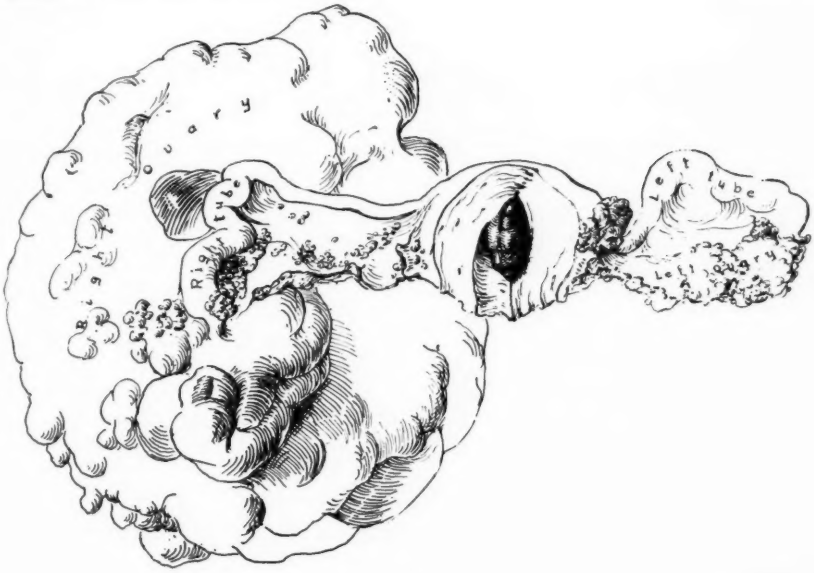
PLATE 91

FIG. 42. Anterior view of the body of the uterus and both tubes and ovaries from a patient (Case 6) with carcinoma of both ovaries and a peritoneal carcinomatosis. The right ovary is replaced by a large carcinoma. The right tube is adherent to the ovarian tumor with the fimbriated end embedded in it. The left ovarian tumor was much smaller than the right one, but was so adherent to the side of the pelvis that only a portion of it was removed. The fimbriae of the left tube are partially embedded in the ovarian tumor of that side. Small carcinomatous implants can be seen on the anterior surface of the right broad ligament and a large one on the anterior surface of the left uterine cornu. A large benign mucosal polyp is present in the uterus. × 3/5.

FIG. 43. A portion of the left ovarian tumor, shown in the preceding illustration, which has invaded the fimbriae of the left tube. At the left the carcinoma is advancing, like a primary growth in this situation, by replacing the epithelium of the mucosal folds. At the right the carcinoma has already partially or completely destroyed some of the mucosal folds. × 25.

FIG. 44. The attempted implantation of cancer cells on a mucosal fold of the fimbriae of the left tube. A clump of cancer cells "a" is resting on the surface of a fold. While the mucosal epithelium beneath the tumor cells is lacking there is no appreciable evidence of any reaction of the tissues of the fold in this situation. Cancer cells "b" are shown attached to a strand of granulation tissue which has arisen on the surface of the fold. The reaction, present here, suggests that this attempted implantation might have taken place. × 130.



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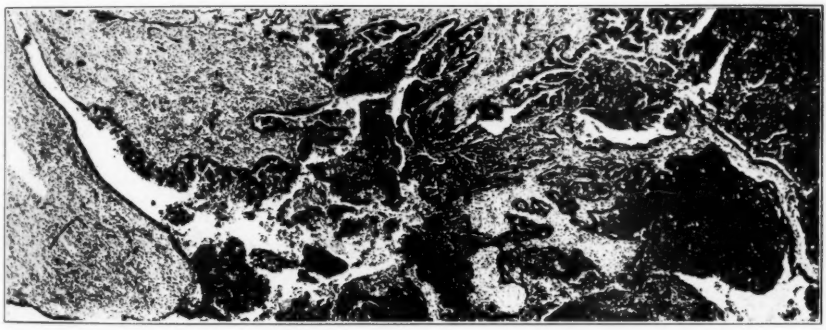

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44

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Implantation Carcinoma of Tubal Mucosa

PLATE 92

FIG. 45. A mucosal fold of the fimbriae of the left tube (Case 6) with a large sessile polypoid growth of granulation tissue on its surface, at the left. This tissue is identical with the tissue frequently found on the surface of the serosa of patients with peritoneal carcinomatosis where it occurs without and also with cancer cells attached to its surface or embedded in it. This tissue forms the stroma of many peritoneal implants and plays an important rôle in their pathogenesis. In the present situation it has developed through breaks in the epithelial covering of the mucosal fold. A small clump of epithelial cells ("a") is included in the base of this tissue. Recognizable cancer cells were not detected in this tissue. However, possible dead cancer cells are present on its surface at "b." At the right a small polypoid growth of newly formed tissue ("c") is present on the surface of the fold. × 54.

FIG. 46. Polypoid granulation tissue on the surface of a mucosal fold, which has developed through a break in the epithelial covering of the fimbriae of the same tube shown in the preceding microphotographs. Cancer cells ("a") are embedded in this tissue. This lesion demonstrates an early stage in one type of implantation of cancer cells on the epithelial covered fimbrial mucosa identical with that often encountered on the surface of the mesothelial covered serosa of patients with peritoneal carcinomatosis (see Figs. 99 and 101). × 54.

FIG. 47. Granulation tissue on the surface of a mucosal fold of the fimbriae from the same series of sections as those shown in the preceding microphotographs. This newly formed tissue has surrounded the carcinoma implanted on the surface of the fimbrial mucosa. At the right of this implant and fused with it more recent granulation tissue has developed. A clump ("a") of viable appearing cancer cells and two clumps ("b") of possible dead cancer cells are embedded in this latter tissue. The last three microphotographs demonstrate that cancer cells escaping from an ovarian carcinoma into the peritoneal cavity may become embedded in granulation tissue arising on the epithelial covered fimbrial mucosa just exactly as they become embedded in similar tissue arising on the mesothelial covered peritoneum. The etiology of this granulation tissue in the two situations should be the same. × 54.

FIG. 48. A mature polypoid carcinomatous implant on the surface of an epiploic appendage, from the same patient as the preceding ones. It presents a stage in the implantation of cancer cells later than the one shown in the preceding microphotograph. × 25.

FIG. 49. A mature, pedunculated, polypoid carcinomatous implant on the serosa of the right tube, also from the same patient. The pathogenesis of these two serosal implants undoubtedly is the same as the pathogenesis of the mucosal implants just demonstrated. × 25.

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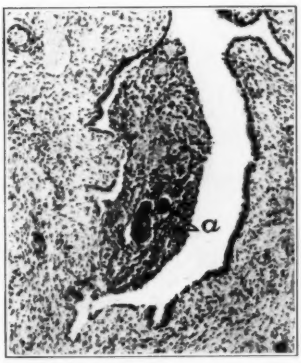

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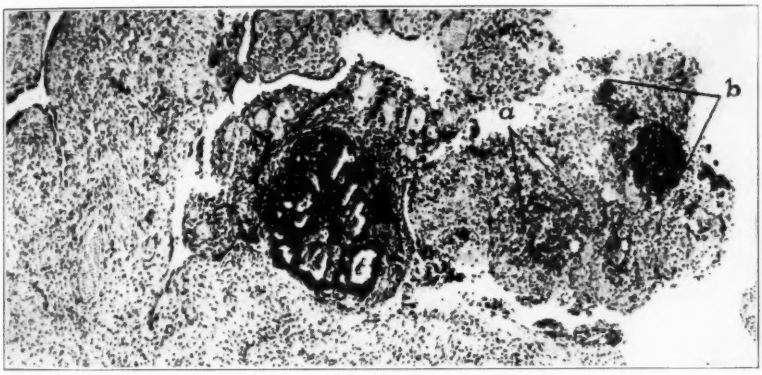

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49

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Implantation Carcinoma of Tubal Mucosa

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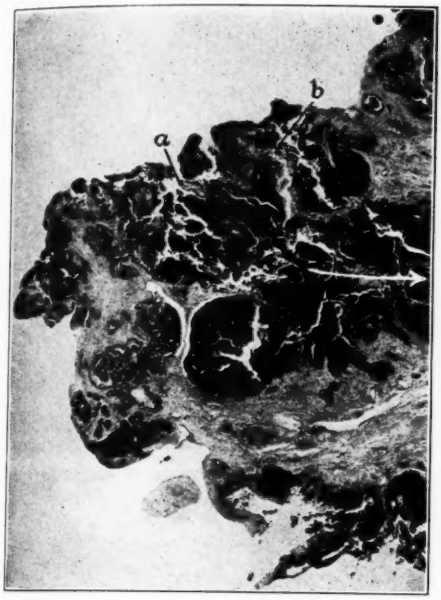
PLATE 93

FIG. 50. Longitudinal section of the distal end of the right tube shown in Figure 42. This end of the tube was embedded in the carcinoma of the right ovary. The ovarian growth has invaded the fimbriated end of the tube from all sides and has replaced nearly all of the mucosal folds of the fimbriae. The intact tips of two mucosal folds appear at "a" and "b." By continuous extension through the abdominal ostium of the tube (see arrow) the carcinoma has invaded and distended the lumen of the ampulla of the tube (see the next microphotograph). × 5.

FIG. 51. A cross section of the ampulla of the tube shown in the preceding section and near the latter. The invasion of the outer portion of the wall of the tube by the ovarian carcinoma is shown at the left. The lumen of this portion of the tube is distended by the tumor which has grown through the abdominal ostium (see the preceding microphotograph). Note that the tumor is penetrating the lumen of the tube by replacing its mucosa and has not invaded the wall of the tube in this situation. × 4.

FIG. 52. An oblique section of the tube demonstrating the continuation of the tumor, shown in the preceding microphotograph, through the lumen of the tube toward the uterus (from a block following the one from which the preceding section was taken). Ovarian carcinoma appears at the bottom of the field where it has invaded the mesosalpinx. At the left the lumen of the tube is partially filled by carcinoma which is a continuation of the growth shown in the preceding section and which has advanced by replacing the tubal mucosa. At the right a still further extension of the growth is indicated. Carcinoma "b" is a continuation of carcinoma "a." Sections of tips of the advancing tumor appear as large emboli of cancer cells in the lumen of the tube. Small true emboli of cancer cells, however, are scattered throughout the lumen of the tube. At "c" isolated carcinoma appears (see the next microphotograph). × 5.

FIG. 53. Higher magnification of the area marked by the pointer "c" in the preceding illustration (section reversed). Carcinoma is shown replacing the tubal mucosa at the right and embedded in granulation tissue at the left. What is the origin of the carcinoma in this situation? See the following five microphotographs, and compare with Figure 47. × 54.



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53

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Implantation Carcinoma of Tubal Mucosa

UNIV. OF MICH.

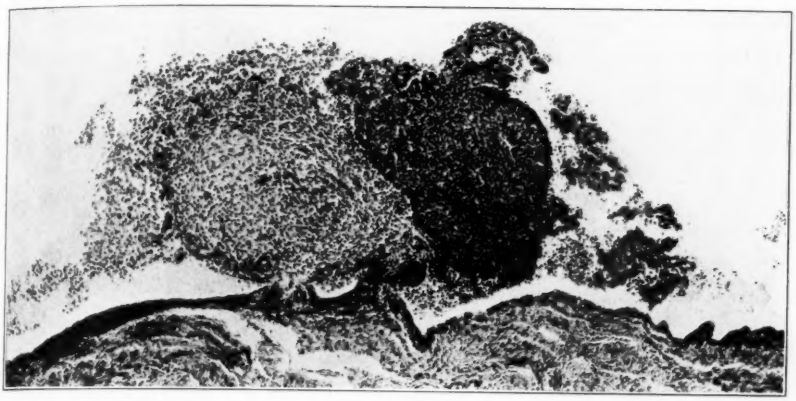
FIG. 54. Another isolated carcinoma of the tubal mucosa from the same series of sections as the ones shown in the two preceding microphotographs. Granulation tissue has developed through breaks in the epithelium of the tubal mucosa similar to that shown in Figure 45 and also similar to the granulation tissue frequently encountered on the serosa of patients with peritoneal carcinomatosis. Evidently cancer cells floating about in the lumen of the tube became attached to the surface of this granulation tissue and have grown here. × 54.

FIG. 55. Isolated carcinoma on the tip of a secondary fold of the tubal mucosa from a portion of the tube nearer the uterus than the implant shown in the preceding microphotograph. The carcinoma in this situation must be derived either from the epithelium of the tubal mucosa (an instance of multicentric origin) or be metastatic from the growth which has invaded the lumen of the tube. The reaction of the tubal mucosa with a loss of its epithelium and a proliferation of the subepithelial tissue cells would furnish a fertile field for the implantation or grafting of cancer cells floating in the lumen of the tube. Compare this microphotograph with the preceding one and also with Figures 33 and 34. The histological picture of the lesions in the different situations is very similar. × 54.

FIG. 56. The same implant pictured in the preceding microphotograph and near the latter in the series of sections. It shows the relation between the tumor cells and the epithelium of the mucosa better than the preceding section. × 54.

FIG. 57. Another implant on a secondary fold of the tubal mucosa. This one is apparently younger than the preceding ones and from the same series of sections. There is an accumulation of lymphocytes in the tissues of the mucosal fold beneath the upper pole of the tumor. This is an evidence of a tissue reaction which may have arisen either before or after the tumor developed. Note that the tumor presents the histological picture of having been added to the mucosa rather than of having arisen from its epithelium. × 54.

FIG. 58. Two implants similar to the preceding one and also from the same series of sections. The larger one is evidently an older implant. The smaller tumor ("a") apparently is a very early one. Although many secondary carcinomas of the tubal mucosa, similar to those shown in the last four microphotographs, were present in this tube, the initial stage of their pathogenesis, which is so well shown in the preceding case, was not detected. × 54.



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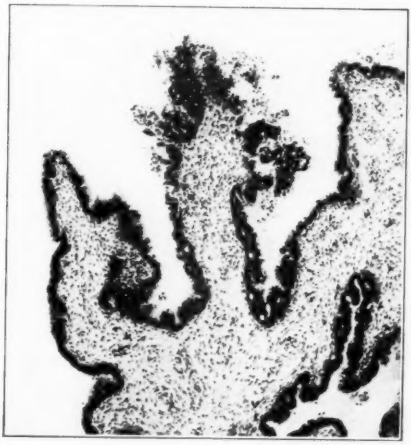

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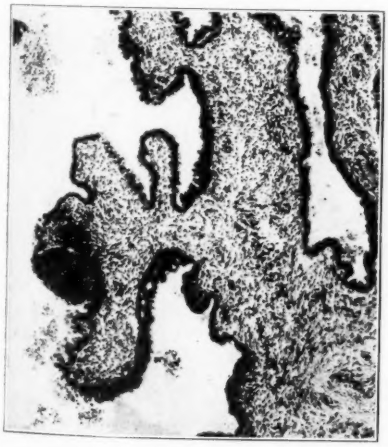

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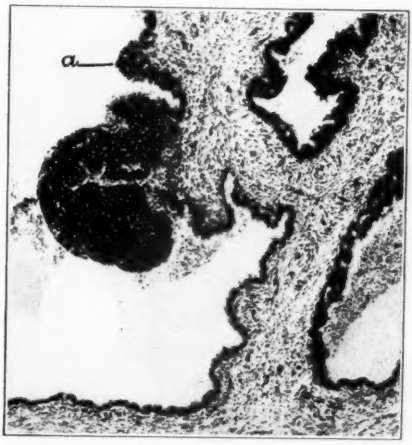

58

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Implantation Carcinoma of Tubal Mucosa

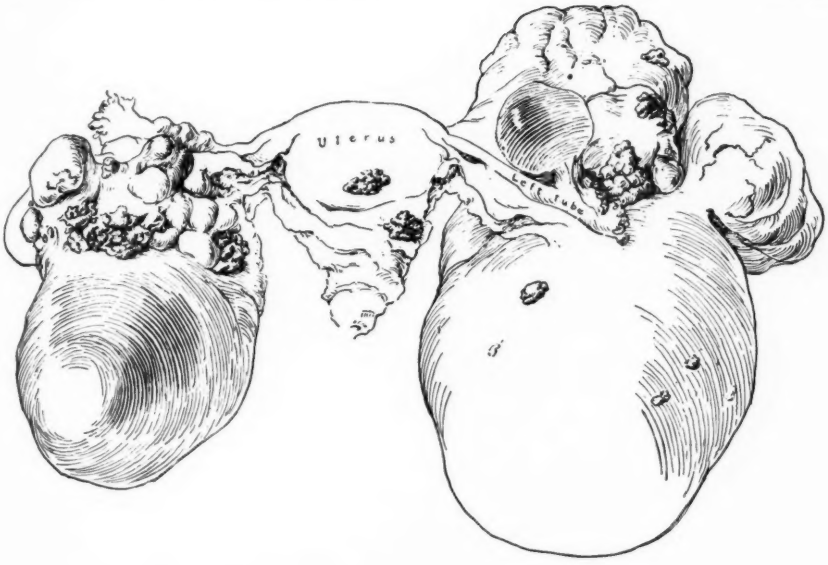
Plate 95

Fig. 59. Anterior view of the uterus, tubes and ovaries from a patient with adenocarcinoma arising in a papillary adenocystoma of both ovaries associated with an extensive peritoneal carcinomatosis (Case 7 of present paper). All stages in the development of various types of polypoid peritoneal implants were encountered in the study of the peritoneal metastases in this case (see Case 4 of previous paper [10]). Newly formed lymphatics were found in the stroma of some of these implants. The gross appearance of this specimen, with implants on the anterior surfaces of the uterus, left broad ligament and ovaries, is shown in this drawing. The fimbriae of the tubes appear normal on casual examination (see Figs. 63 and 69). × 2/3.

Fig. 60. Section of sediment obtained by centrifugalizing some of the ascitic fluid removed from the patient with the ovarian carcinomas shown in the preceding illustration. The clumps of deeply stained cancer cells are easily discernible. Cells similar to these might become implanted on the peritoneum and the fimbrial mucosa if conditions favorable for this phenomenon arose in these situations (see Figs. 61, 62 and 65). × 130.

Fig. 61. The tip of a polypoid outgrowth of early granulation tissue which has arisen on the surface of the vesico-uterine reflection of peritoneum from Case 7. A portion of this peritoneum was excised and placed in formalin before proceeding with the major part of the operation. Clumps of cancer cells, similar to those shown in the preceding microphotograph, are becoming attached to and enmeshed in the tip of this tissue, its youngest and most actively growing portion. They are not found in any other portion of this outgrowth of granulation tissue. × 54.

Fig. 62. Another outgrowth of granulation tissue similar in situation and form to the preceding one and possibly of the same age. It presents a stage in this type of cancer cell implantation a little later than the one just shown. This granulation tissue arose from the serosa in response to stimulation caused, in some way, by cancer cells which had escaped into the peritoneal cavity. The granulation tissue in both outgrowths is abundantly supplied with newly formed blood vessels. Yet, in spite of a careful study of many sections of these two outgrowths, lymph vessels cannot be recognized in them. However, they may be present. × 54.



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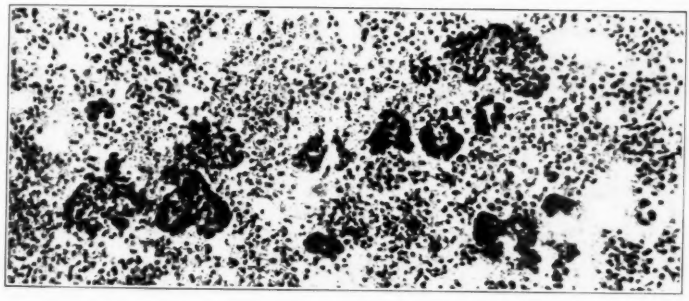

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62

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Implantation Carcinoma of Tubal Mucosa

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FIG. 63. The fimbriae of the right tube shown in Figure 59. A careful examination of this tube under water, with a hand lens, demonstrated three small nodules, "a," "b" and "c," which are attached to the mucosal folds of the fimbriae and resemble very closely many of the polypoid implants on the peritoneum of this and other patients with peritoneal carcinomatosis of ovarian origin. These nodules might easily be missed in a casual inspection of the fimbriae (see Fig. 59). × 1½.

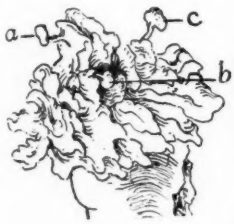
FIG. 64. Longitudinal section of the fimbriated end of the tube shown in the preceding illustration. A small portion of a pedunculated polypoid implant is indicated by the letter "a," a sessile polypoid implant by "b" and a pedunculated one by "c." A portion of the slender pedicle of implant "c" can be seen pointing upward and to the right. These implants correspond to the three nodules shown in the preceding illustration. × 5.

FIG. 65. A pedunculated polypoid outgrowth of granulation tissue, with clumps of cancer cells becoming implanted in it, is shown between two mucosal folds of the fimbriae. This implant is marked "a" in the preceding microphotograph but from another section in this series. This outgrowth of granulation tissue arose from the subepithelial tissues of a mucosal fold of the fimbriae just as the polypoid outgrowths of granulation tissue shown in Figures 61 and 62 arose from the submesothelial tissues of the serosa of the vesico-uterine fold of peritoneum in the same patient. The pathogenesis of the granulation tissue in the two situations is undoubtedly the same. Cancer cells, floating about in the ascitic fluid present in this patient, are becoming implanted in newly formed granulation tissue in both situations. Portions of the slender pedicle of this outgrowth of granulation tissue can be seen extending downward toward the mucosal fold below it (see Fig. 67). × 25.

FIG. 66. Higher magnification of polypoid implant "b" shown in Figure 64 (from the same section) and also of the mucosal fold to which it is attached. This implant is sessile and much older than the one shown in the preceding microphotograph. × 25.

FIG. 67. A cross section ("a") of the pedicle of the polypoid outgrowth of granulation tissue shown in Figure 65 (from another section in this series) and a portion of the underlying mucosal fold from which it arises. Blood vessels can be seen readily in the cross section of this pedicle. Lymph vessels can not be detected in it although they may be present. They are very evident in the adjacent fimbrial mucosa from which the granulation tissue arose. × 54.

FIG. 68. A higher magnification of implant "c" seen in Figure 64 (from another section in this series) including a cross section ("a") of its long slender pedicle and the tip of the mucosal fold from which the pedicle arises, below it and to the right of the body of the implant. Blood vessels can be seen readily, especially under higher magnification, in this pedicle, but lymph vessels can not be detected in it. Cross sections of these pedicles are very characteristic and resemble cross sections of small umbilical cords. It is obvious that this implant represents a later stage of the process shown in Figure 65. × 54.



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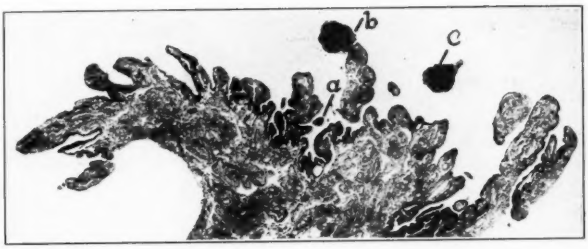

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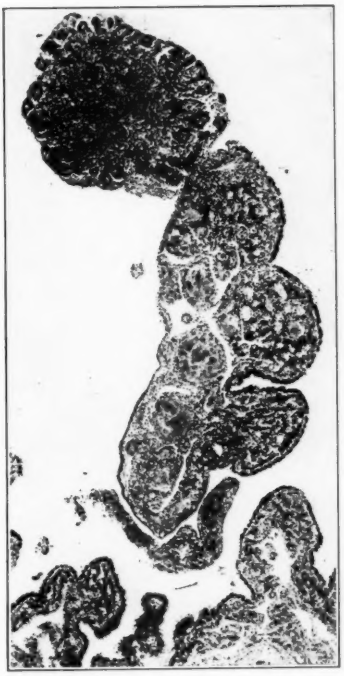

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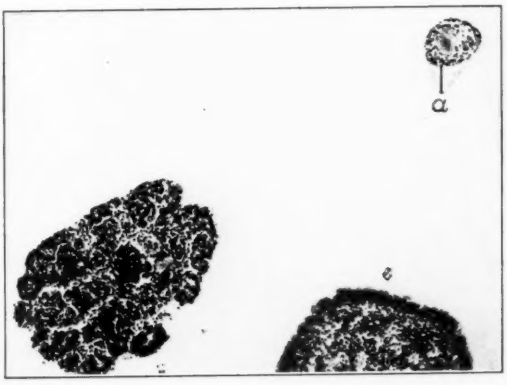

68

Sampson

Implantation Carcinoma of Tubal Mucosa

UNIV. OF MICH.

PLATE 97

FIG. 69. The distal end of the left tube illustrated in Figure 59. A pedunculated polypoid implant ("a") is shown attached to the serosa of the ampulla of the tube. A similar implant ("b") is attached to the mucosa of the fimbriae. Implant "a" was injured in blocking the tube. Sections of a polypoid serosal implant similar to implant "a" and from the same patient are shown in Figures 60 to 62 inclusive of a previous paper.[10] × 1½.

FIG. 70. Longitudinal section of the fimbriated end of the tube shown in the preceding illustration (compare with Fig. 64). The mucosal implant "b" of Figure 69 appears for its entire length, including its long slender vascular pedicle and the attachment of the latter to the mucosa of the fimbriae. × 5.

FIG. 71. Higher magnification of the lower portion of the body of the implant and a portion of its pedicle shown in the preceding microphotograph. Blood vessels filled with blood may be followed, in this series of sections, from preexisting blood vessels of the fimbriae through the entire length of the pedicle up into and throughout the stroma of the implant proper. Lymph vessels, with interruptions, may be followed in like manner from those in the fimbrial mucosa through the pedicle and into the base of the implant proper. However, these cannot be detected in the dense tissues of the latter. Lymph vessels or branches of the same vessel are marked by the pointers "a," "b" and "c." A few lymphocytes are present in the lumens of vessels "b" and "c." Carcinoma is present between and to the right of the upper portion of these two vessels and later might have invaded these vessels and gained access to the lymph channels of the fimbriae to which the pedicle is attached if the patient had not been operated upon. × 54.

FIG. 72. Higher magnification of a portion of the fimbrial mucosa at the attachment of the pedicle of the above described implant (see Fig. 70) but from another section in this series. Only a small portion of the pedicle appears in this section. A judged partially collapsed lymph vessel ("a") can be just detected in this portion of the pedicle. Blood vessels cut obliquely and longitudinally can be seen above the lymph vessel. There was no difficulty in following the blood vessels, easily seen in the series of sections, from preexisting blood vessels of the mucosa into and through the pedicle of the implant and into its body. However it is impossible to establish the continuity of the judged lymph vessels of the pedicle with the nearby, easily discernible lymph vessels of the fimbrial mucosa. This difficulty is frequently encountered in the study of the origin of judged newly formed lymph vessels in polypoid implants since non-injected lymph vessels, when collapsed, cannot be detected. × 25.

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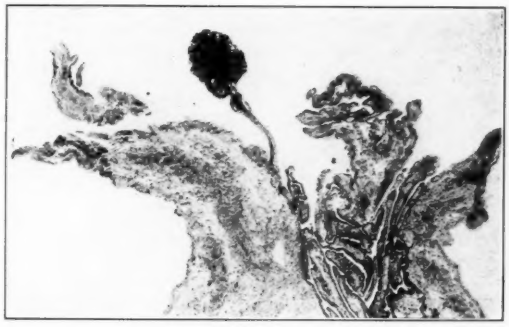

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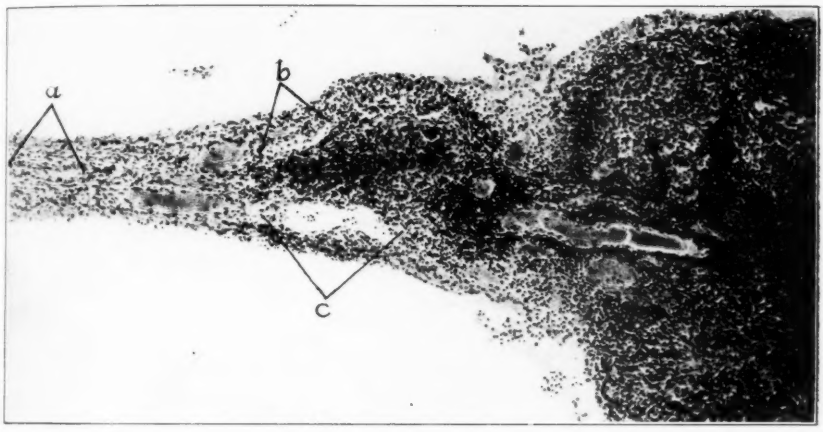

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PLATE 98

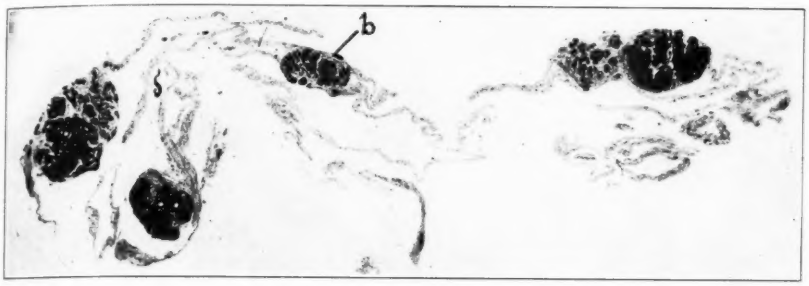
FIG. 73. Cobweb-like adhesions with metastatic carcinomas enmeshed in them like flies in a spider's web, from a patient (Case 8) with adenocarcinoma of both ovaries and a peritoneal carcinomatosis. These adhesions, which extended from the right tube to the suspensory ligament of the ovary, were carefully removed at the onset of the operation and immediately placed in formalin. The implants, shown here, are of nearly the same size and therefore may be of approximately the same age. However, in other sections of these adhesions all stages in the pathogenesis of these implants are encountered. × 5.

FIG. 74. An early implantation of cancer cells in the adhesions shown in the preceding microphotograph and from the same series of sections. A growing clump of cancer cells is covered by granulation tissue which has arched over them. × 54.

FIG. 75. A clump of viable appearing cancer cells caught in a loop of a strand of granulation tissue, also from the same series of sections as the preceding ones. × 130.

FIG. 76. A very early stage in the implantation of cancer cells in a loop of granulation tissue similar to, but more vascular than, the preceding tissue. This stage is earlier than the one shown in Figure 74, also from the same series of sections. Cells from the granulation tissue beneath the carcinoma have invaded the fibrin holding the latter in place (compare with Figs. 36 and 37 showing a similar stage in the implantation of cancer cells on the tubal mucosa). × 130.

FIG. 77. A judged early stage in the implantation of cancer cells on the mucosa of the ampulla of the right tube of Case 8. This area corresponds to the one marked by pointer "c" in Figure 85, from a nearby section in this series. Here the epithelium is lacking and viable appearing cancer cells are covered by fibrin which contains wandering cells from the subepithelial tissues of the mucosa. Compare with the implantation on serosal adhesions shown in the preceding microphotograph and also with Figures 36 and 37. The process apparently is the same in all four situations. Carcinoma once established in the tubal mucosa would be in close proximity to the mucosal lymph vessels and might easily invade these vessels and thus spread in the lymphatic circulation of the tube. × 130.



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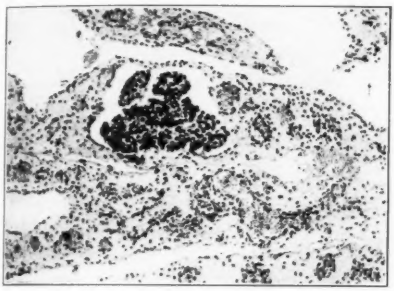

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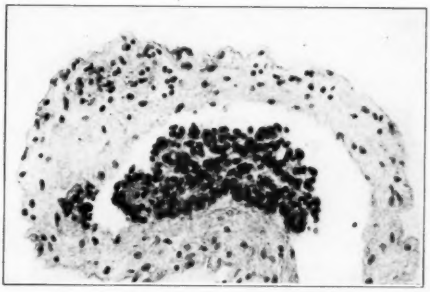

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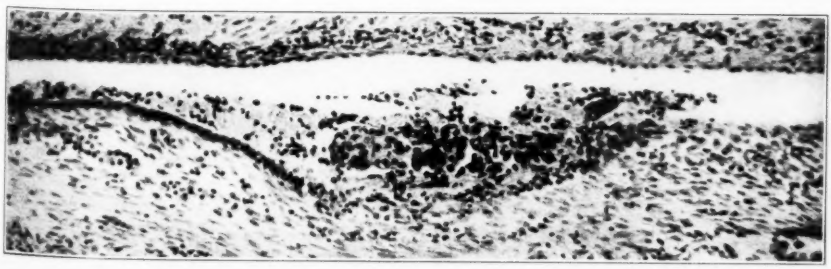

77

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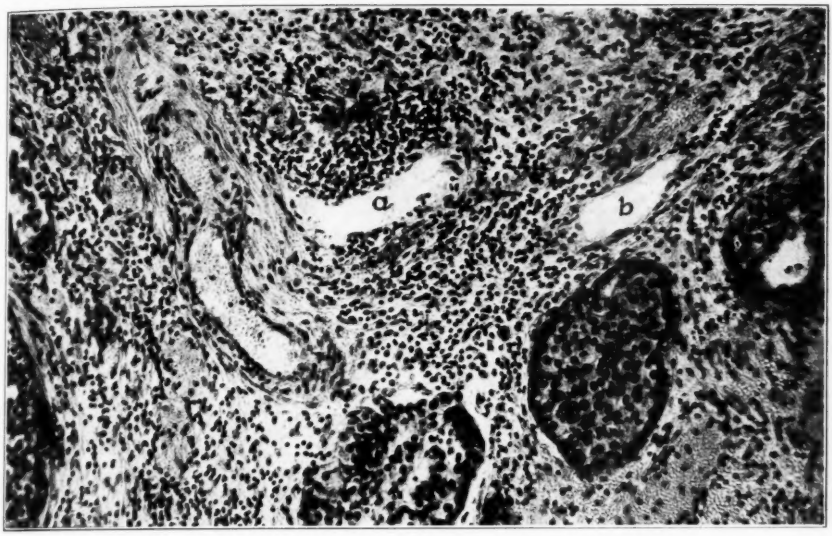
PLATE 99

FIG. 78. A portion of a large implant in the adhesions shown in Figure 73, from another section of this series. Two judged lymph vessels or portions of the same vessel are marked "a" and "b." They can be easily distinguished from the blood vessels since the latter are filled with blood. It is impossible to determine whether or not carcinoma has invaded any of the lymph vessels in these adhesions. Some of the lymph vessels may have been invaded by carcinoma. × 130.

FIGS. 79 and 80. Mature portions of the adhesions pictured in the preceding microphotographs showing easily recognized newly formed blood vessels and spaces "a" and "b" lined by endothelium-like cells without blood in their lumens. They are probably lymph vessels. Their continuity with preexisting lymph vessels of the tube or suspensory ligament of the ovary cannot be established since the adhesions were removed at the beginning of the operation. × 54.

FIG. 81. A portion of a strand of newly formed tissue, similar to the adhesions just pictured, which has arched over the surface of the left tube, from the same patient. This strand contains newly formed blood vessels which are accompanied by a newly formed lymph vessel. The origin of the latter from a preexisting lymph vessel of the tubal wall can be seen plainly in this attachment of the strand to the serosa of the tube. The lymph vessel, shown above, extends the entire length of the strand and is continuous with a similar vessel in the attachment of the other end of the strand to the serosa of the tube (see Figs. 127 and 128 of a previous paper [10]). Carcinoma is not present in this newly formed tissue which forms the stroma of many implants. If it had been present it might have invaded the nearby lymph vessels. If it had been present in the preexisting lymph vessels of the tube beneath the attachment of the newly formed tissue it might have spread into the newly formed lymph vessels. This phenomenon has been demonstrated by the writer (see Figs. 129 to 131 inclusive of a previous paper [10]). × 130.



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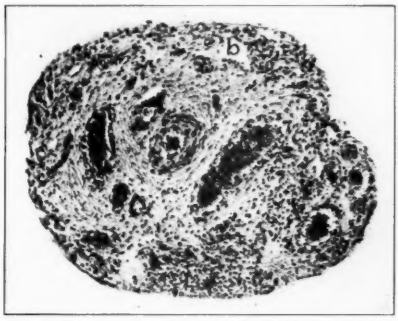

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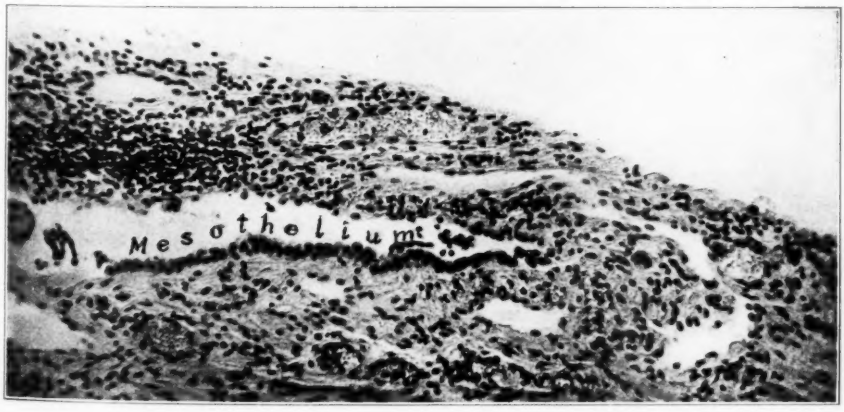

81

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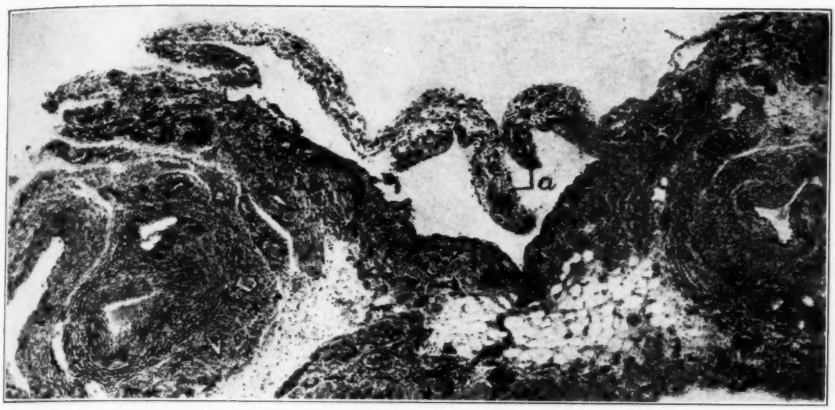
FIG. 82. A portion of the left mesosalpinx showing a strand of newly formed tissue which has arched over its surface similar to the strand shown in Figure 81, from the same patient. Both attachments of this strand to the serosa of the mesosalpinx appear in this section. Lymph vessels (possibly one vessel) are easily discernible (under higher magnification) throughout the entire length of the strand just as they are discernible in the strand in the preceding microphotograph. Cancer cells are present in the lumen of this vessel in all portions of the strand. I am unable to determine whether the growth of the carcinoma in the lymphatics is continuous throughout its entire length or is both continuous and interrupted (embolic). In the field shown in this microphotograph carcinoma is present in only the newly formed lymph vessel of the strand and in the preexisting lymph vessel of the subserosa from which the newly formed vessel arose (see the following microphotograph). However, in other portions of this section carcinoma is present both in large lymph vessels of the mesosalpinx and in its subserosal lymphatics. × 10.

FIG. 83. Higher magnification of the right-hand portion of the strand of newly formed tissue and the mesosalpinx from which it arose, shown in the preceding microphotograph. Carcinoma is present in both the lumen of the newly formed lymph vessel and the lumen of the preexisting lymph vessel of the subserosa. The study of other sections in the series demonstrated that both the lymph vessel and the carcinoma in it in the strand of newly formed tissue are continuous with the lymph vessel and the carcinoma in the subserosa. Since carcinoma is present in both the deep and the subserosal lymph vessels of other portions of the mesosalpinx in this section and in other sections of the series, it is natural to assume that the carcinoma in the lymph vessel of the newly formed tissue reached its present situation by either lymphatic permeation or metastasis from the growth in the preexisting lymph vessels of the mesosalpinx (compare with Figs. 95 and 96). This field is an excellent example of peritoneal carcinomatosis of lymphatic origin. × 130.

FIG. 84. Higher magnification of the loop of the strand of newly formed tissue indicated by "a" in Figure 82, from a nearby section in this series. This print is mounted at right angles to the one shown in Figure 82. The carcinoma in the upper arm of the loop is in a lymph vessel and is continuous with the carcinoma shown in the preceding microphotograph. The three clumps of judged cancer cells, below the carcinoma filled lymph vessel, are situated in the angle of a loop of newly formed tissue, similar to those shown in Figure 75. In spite of a careful study of the sections covering this field we were unable to establish a continuity between the carcinoma in the lymph vessel of the loop and that in the angle of the loop. I am not sure whether the clump of cells marked "a" is composed of cancer or mesothelial cells. Note that their nuclei are not as large as those of the cells in the clumps above them. × 130.



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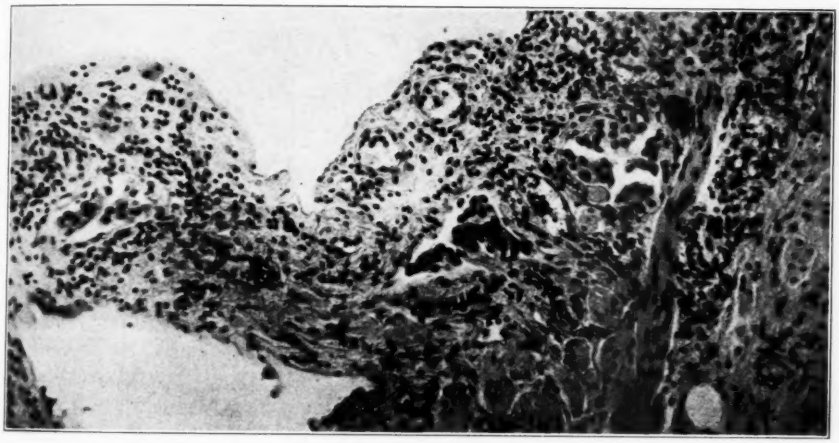

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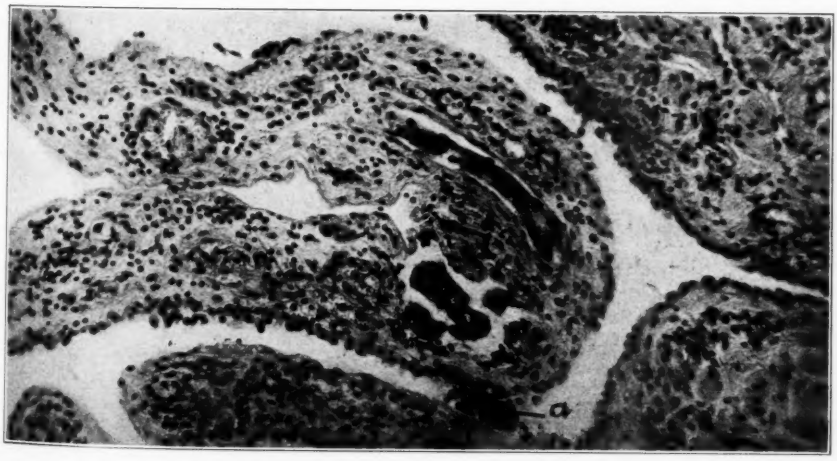

84

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Implantation Carcinoma of Tubal Mucosa

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Plate 101

Fig. 85. Longitudinal section of the distal end of the right tube and the ovarian carcinoma beneath it (Case 8). The tube is stretched over the carcinoma which has grown ("a") over the surface of the elongated lower lip of the fimbriae. A metastasis ("b") with the histological structure of a polypoid implant has arisen from the fimbriae representing the upper lip of the abdominal ostium of the tube. From its situation it must have arisen on the surface of a portion of the fimbriae covered with epithelium. The judged early implantation of cancer cells on the ampullar mucosa of Figure 77 is situated in a nearby section of this series in an area corresponding to that indicated by "c." × 5.

Figs. 86 and 87. Higher magnification of implant "b" of the preceding illustration and also of implant "b" of Figure 73. They both have a similar structure and probably a like pathogenesis. Compare with metastases of judged lymphatic origin shown in Figures 90 and 91. × 25.

Fig. 88. Longitudinal section of the distal end of the left tube including its fimbriae proper, the ovarian fimbriae at the right and a portion of the mesosalpinx. Even under this low magnification the metastases "a" in mucosal folds of the fimbriae do not resemble the one marked "b" in Figure 85, from the same patient. Carcinoma is present in lymph vessels of the fimbrial mucosa and of the mesosalpinx. For a higher magnification of the two mucosal tumors, marked "a," see Figure 90. A patch of endometriosis on the mesosalpinx is marked "End." × 5.

Fig. 89. A section similar to the preceding one and from the same series of sections. A mucosal fold of the ovarian fimbriae, to the right, is greatly enlarged and distorted by the tumor (compare with Fig. 22). Carcinoma can be seen under higher magnification in the lymph vessels of the mucosal folds. The pathogenesis of the mucosal carcinomas shown in the last two microphotographs was judged to be by permeation and metastasis from the ovarian tumor through the lymph vessels. × 5.

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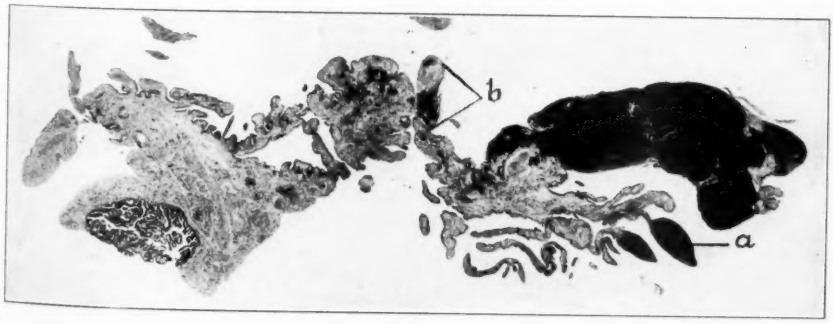

89

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FIG. 90. Higher magnification of the two mucosal folds marked by pointer "a" in Figure 88. Carcinoma is present in lymph vessels in the base of the first mucosal fold. The distal portion of both folds appears to have been distended by a growth which has reached and invaded their tissues from within. Compare with the metastases shown in Figures 66 and 70 where the metastatic tumors consist of the growth of cancer cells in newly formed tissue arising on the surface of a mucosal fold. × 25.

FIG. 91. Higher magnification of the carcinoma in the mucosal fold marked "a" in Figure 89. In some ways the condition shown here resembles a pedunculated polypoid implant. The apparent pedicle is a non-distended portion of a mucosal fold, as in the two folds in the preceding microphotograph, and does not consist of newly formed tissue like the pedicles of the mucosal implants shown in Figures 67 and 68. × 25.

FIG. 92. Carcinoma in a mucosal fold of the ampulla of the left tube, the fimbriae of which are shown in the four preceding microphotographs. Here the fold is distended by carcinoma which has invaded it from within and which apparently has grown in lymphatics. Carcinoma has even forced its way from a lymphatic through the overlying epithelium into the lumen of the tube (see "a"). This phenomenon is not of infrequent occurrence in situations where the mucosal lymph vessels are filled with a growing carcinoma (see Figs. 16 and 17). The anatomical basis for such a phenomenon may be seen in Figure 8. Carcinoma is present in lymph vessels in all portions of this tube including its fimbriae and in the mesosalpinx. Since no portal of entry for the carcinoma into the lymphatic circulation was discovered other than the ovarian tumor it may be reasonably assumed that the tumors of this tube were secondary to the ovarian by permeation and metastases through the lymph vessels. × 54.

FIG. 93. Cancer cells ("a") becoming embedded in granulation tissue which has developed on the surface of a mucosal fold near the two folds marked "a" in Figure 88, from another section in this series. This granulation tissue is in the form of a polyp with a long slender vascular pedicle attached to a fold, the tip of which is shown at the extreme left of this microphotograph. Lymph vessels can be seen accompanying the blood vessels in the fold beneath the attachment of the pedicle (not shown in this microphotograph). The phenomenon shown here represents a stage in the implantation of cancer cells in granulation tissue arising from tubal fimbriae earlier than that shown in Figure 65. Carcinoma in a lymph vessel of an adjacent fold is marked "b." Carcinoma has developed in the fimbrial mucosa of this tube in two ways: one through lymph channels from the primary ovarian carcinoma; and the other by cancer cells escaping into the peritoneal cavity from the ovarian carcinoma and becoming implanted in granulation tissue which has developed on the surface of the fimbriae. Another implantation of carcinoma, similar to this one, was found in this specimen. × 54.

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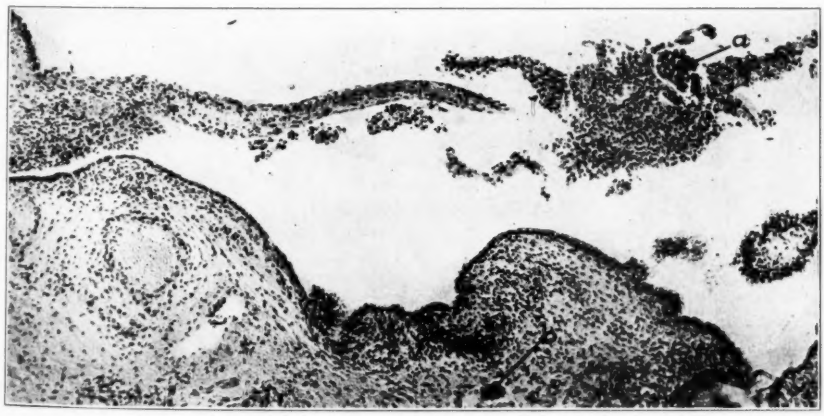

93

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Implantation Carcinoma of Tubal Mucosa

Plate 103

Fig. 94. A portion of the mucosal fold, marked "b" in Figure 89, but from another section in this series, with a sessile polypoid mass of early granulation tissue on its surface. Clumps of cancer cells ("a") are in a space in this tissue which resembles the lumen of a lymph vessel. Carcinomas "b" and "c" are shown in preexisting lymph vessels of the mucosal fold. × 54.

Fig. 95. Higher magnification of the granulation tissue and a portion of the mucosal fold beneath it shown in the preceding microphotograph, from a nearby section in this series. The carcinoma in a judged newly formed lymph vessel in the granulation tissue of the previous section is also shown here. A small lymph vessel below a blood vessel is marked by "b." The preexisting lymph vessel with carcinoma in its lumen below the base of the granulation tissue is shown in the series of sections to be continuous with mucosal lymph vessel "b" of the preceding section. × 130.

Fig. 96. The same granulation tissue shown in the preceding microphotographs, from a nearby section in this series. In this section the newly formed lymph vessel ("a"), which contains carcinoma in the previous sections, is shown to be continuous with the preexisting lymph vessel ("b") of the mucosa beneath the granulation tissue. It is evident, by comparing the last three microphotographs, that the carcinoma in a preexisting mucosal lymph vessel, in this instance, has spread into the newly formed lymph vessel, derived from it, in the granulation tissue. Compare with Figure 83 where the same phenomenon has occurred in granulation tissue arising on the serosa. I believe that carcinoma "c" is also in a lymph vessel. Since carcinoma frequently becomes implanted in granulation tissue it must also be evident that the carcinoma of the implant may invade a nearby newly formed lymph vessel, if the latter is present, and then spread to the preexisting lymphatics of the tubal mucosa. The implanted carcinoma may be small and not recognized as such. It is also true that carcinoma in a lymph vessel similar to the carcinoma shown here may penetrate the wall of the vessel and invade the tissue about it. Although there is no evidence of implanted carcinoma in this granulation tissue, it is present in granulation tissue in other situations of the fimbrial mucosa in this case (see Fig. 93). Eventually cancer cells might have become implanted in this granulation tissue if the operation had been deferred. Therefore, even if carcinoma in the stroma of a metastasis on the tubal mucosa can be shown to be continuous with carcinoma in a newly formed lymph vessel, it may be impossible to ascertain whether implanted carcinoma had invaded the lymph vessel, or carcinoma in the lymph vessel had invaded the tissue about it. × 130.

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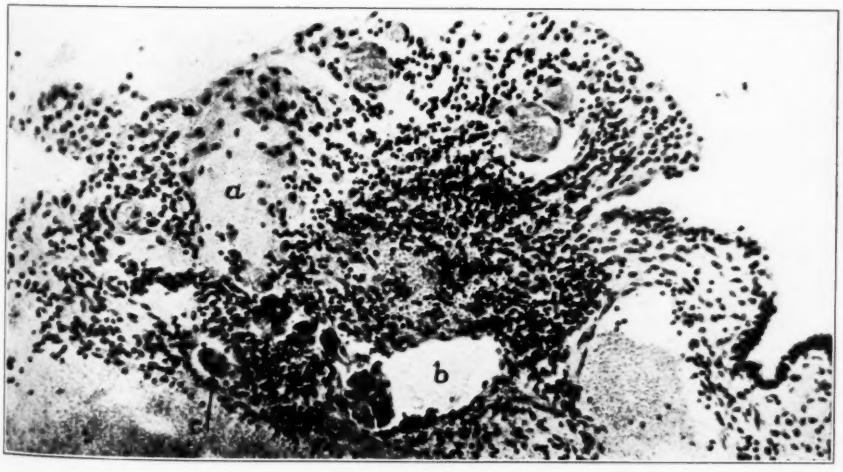

96

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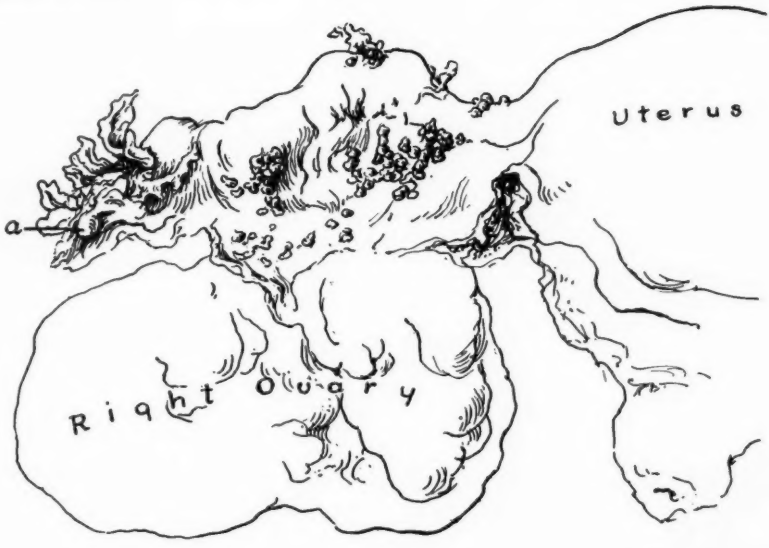
FIG. 97. Right ovary, tube and a portion of the uterus from a patient (Case 9) with carcinoma of both ovaries and an associated peritoneal carcinomatosis. Peritoneal implants, here, can be seen on the surface of the tube and mesosalpinx. Similar metastases were found on the surfaces of many other pelvic structures, including the sigmoid with its epiploic appendages (see Fig. 83 of previous paper[10]). At the operation the ovarian tumor was found to be adherent to the posterior surface of the uterus and had invaded that organ in this situation. A small nodule ("a") similar to the fimbrial implants shown in Figures 63 and 69 is attached to the tips of the mucosal folds of the fimbriae which appear to be fused at the base of the nodule. The left ovarian tumor was similar but smaller than the right. The left tube appeared normal except for a few implants on its serosa. Its fimbriae were grossly and histologically normal. Clumps of cancer cells, similar to those found in the ascitic fluid, were present in the lumens of both tubes and were much more numerous in the left tube (see Figs. 108, 109 and 112). Natural size.

FIG. 98. A very early implantation of cancer cells on the surface of the tube shown in the preceding illustration. The sections of the tube covering this area are sufficiently close together to demonstrate that the carcinoma in this situation is isolated and does not represent the advancing portion of a large growth. × 54.

FIG. 99. Two early implantations of cancer cells ("a") in polypoid granulation tissue arising on the serosa of the tube shown in the preceding microphotograph. × 54.

FIG. 100. A portion of a mature polypoid implant attached to the serosa of the same tube as the preceding ones. The condition, shown here, presents a stage in the life history of this type of implant later than that illustrated in the preceding microphotograph. × 54.

FIG. 101. A cluster of implants of different ages on the serosa of the tube shown in the preceding microphotographs. To the right of the center is a sessile polypoid metastatic tumor which I believe is of implantation origin and well may represent a later stage of a condition similar to that shown in Figure 98. All stages in the development of implantation metastases, histologically similar to this one, were found on an epiploic appendage from this patient (see case report). Carcinoma is present in judged lymph vessels ("a") of the tubal wall and could well have come from the spread of the carcinoma above them. This is not an infrequent phenomenon in patients with peritoneal carcinomatosis. Attached to the upper surface of the mature sessile implant is a polypoid mass of fibrin with a clump of cancer cells ("b") enmeshed in its tip. The replacement of this fibrin by granulation tissue would give rise to a condition similar to the early polypoid granulation tissue at the left with carcinoma ("c") embedded in it. Beneath the latter is a still later stage in the life history of these implants a little earlier than the one in Figure 100. A portion of a sessile polypoid implant, granulation tissue stage, appears at the extreme right-hand portion of the microphotograph. Compare with Figure 107 showing a cluster of judged similar implants, also of different ages, on the fimbrial mucosa of the same tube. × 54.



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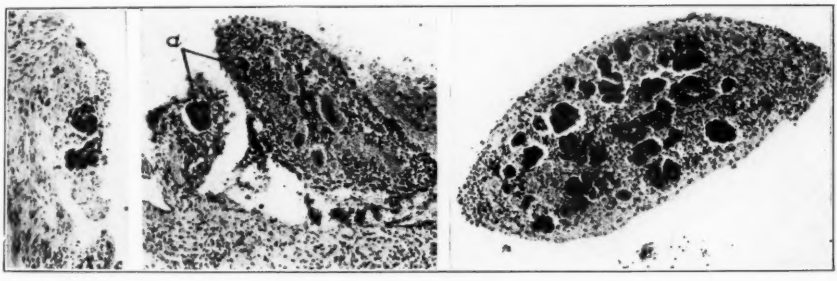

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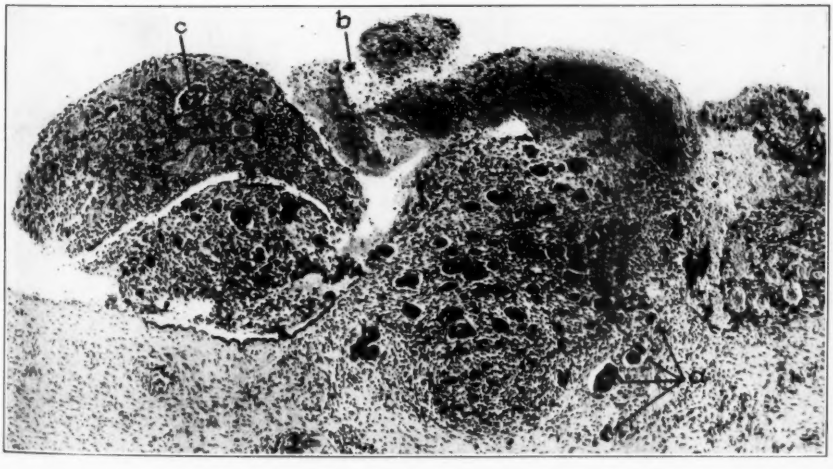

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FIG. 102. Longitudinal section of the distal end of the right tube, shown in Figure 97, in a plane that includes the fimbriae with the nodule indicated by "a" of that illustration, a portion of the mesosalpinx, and the abdominal ostium ("b") of the tube cut obliquely. The nodule "a" of Figure 97 can be seen in the center of the fimbriae. It appears as a darkly stained multinodular rounded tumor, with a constricted base, attached to the tips of mucosal folds which are fused together. This tumor points upward and to the left. At the right of its base is another tumor of about the same size, caused by the fusion of the tips of the mucosal folds in this situation (see Fig. 107). The fimbrial mucosa at the left appears normal. Carcinoma is not present in its lymph vessels. The lymph vessels of the fimbrial folds at the right are injected with carcinoma (see also Fig. 105), which apparently comes from the extension of the growth in lymph vessels at the base of and between the folds. The latter lymph vessels, filled with carcinoma, can be detected, even under this low magnification, in the mucosa between the fimbrial folds just described and the fimbrial tumors. It would appear that the carcinoma in the fimbrial folds at the right and in the fimbrial tumors might be closely related. Small emboli of cancer cells can be seen, under higher magnification, in lymph vessels indicated by the pointers "c" and "d." A higher magnification of the mucosal fold, marked by the arrow, is shown in Figure 106. × 5.

FIG. 103. Higher magnification of the pedunculated polypoid implant on the serosa of the mesosalpinx marked "a" in the preceding microphotograph. It is a very mature serosal implant with a long tortuous pedicle. Implants similar to this one are not infrequent in patients with peritoneal carcinomatosis. Cross sections of the pedicle appear above the body of the implant. Cancer cells are present in a judged lymph vessel ("a") of the pedicle. These cells could well come from the implant and represent the attempted spread of the carcinoma in the implant into the lymph vessels of the mesosalpinx. Carcinoma was not found in the lymph vessels of the mesosalpinx beneath this implant. Cancer cells ("b" and "c") apparently are becoming embedded in newly formed tissue which recently has arisen on the surface of the implant (compare with Fig. 98). In this manner implants frequently increase in size. × 54.

FIG. 104. Higher magnification of a portion of the normal appearing fimbrial fold shown at the left in Figure 102. The pattern of the lymph vessels in this fold is well illustrated. × 54.

FIG. 105. Higher magnification of the fimbrial folds shown at the right in Figure 102. The lymph vessels of these mucosal folds (compare with those in the preceding microphotograph) are filled with carcinoma as with an injection mass. Carcinoma is also present in lymph vessels of the mucosa at the base of and between the folds. Lymphatic metastases or permeation from the ovarian tumor, which in this case had invaded the lymph vessels of the ovary, would be an acceptable explanation for the carcinoma in this situation. The possibility of the origin of the carcinoma in the lymph vessels from the spread of the secondary mucosal tumors of the fimbriae shown in Figure 102 also must be considered (see Fig. 107). × 54.

102

103

104

105

Sampson

Implantation Carcinoma of Tubal Mucosa

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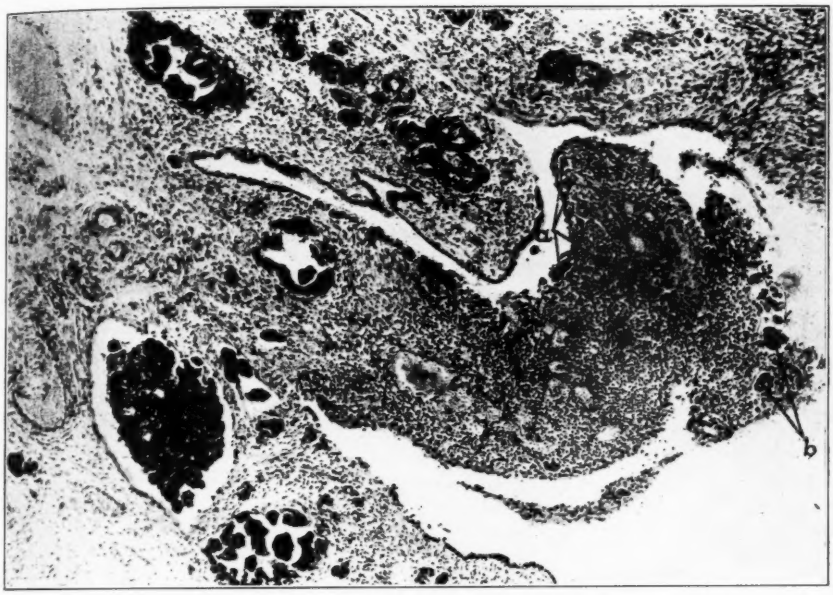
FIG. 106. Higher magnification of the mucosal fold of the fimbriae marked by the arrow in Figure 102. A polypoid mass of early granulation tissue has arisen from the tip of this fold. Clumps of cancer cells ("a") are partially or completely embedded in the distal portion of this tissue. Compare with similar conditions arising on the serosa of the same tube shown in Figures 99 and 101. Note the implantation of clumps of cancer cells ("b") in a younger portion of this tissue similar to that shown arising not only on the tubal serosa of this case (see Figs. 98 and 103) but also on the fimbrial mucosa of other cases (see Figs. 30, 47, 65 and 93). The cancer cells, just described, undoubtedly came from the ascitic fluid in which the fimbriae were immersed (see Fig. 108). The carcinoma in the lymph vessels of the base of this fold and in the adjacent fimbrial mucosa clearly antedates the cancer cells implanted in the granulation tissue of the fold. The carcinoma in the lymph vessels must have arisen by permeation or embolism either primarily from the ovarian growth or from the secondary mucosal neoplasms shown in Figure 102. Granulation tissue like this, arising on adjacent folds, would cause the fusion of the folds shown in the next microphotograph. × 54.

FIG. 107. Higher magnification of the tumor or tumors attached to the fused tips of mucosal folds of the fimbriae shown in Figure 102. How may one account for the condition shown here other than by permeation or metastasis through the lymph vessels from the primary ovarian tumor? Above the center of the microphotograph and a little to the left is situated a multinodular conglomerate tumor which consists of polypoid outgrowths of judged newly formed tissue of various ages containing carcinoma (similar to those shown in Figs. 99, 100 and 101), which are either fused with or clustered about a central tumor. The amount of carcinoma in each outgrowth varies, as in the latter situations, with the age of the outgrowth. Outgrowth "a," which consists of very early granulation tissue, contains only a few small clumps of cancer cells implanted on its surface and embedded in it (compare with the preceding microphotograph). In the polypoid newly formed tissues, "b" and "c," which appear in cross section, the stroma not only is older than that in "a," but it contains a correspondingly greater amount of carcinoma (compare with Fig. 101). The amount of carcinoma in the polypoid outgrowth of newly formed tissue "d" is still greater than in the preceding ones (compare with Fig. 100). The conditions just described are similar to the various stages in the pathogenesis of polypoid implants on the serosa of patients with peritoneal carcinomatosis (see Fig. 101 showing a cluster of implants of various ages on the serosa of the same tube). In this section similar polypoid outgrowths are clustered about or fused with a central polypoid outgrowth similar but larger. This central tumor is attached by a constricted base or pedicle to the fused mucosal folds beneath it. Since the conglomerate tumor has grown by the fusion of probable multiple polypoid implants of various ages, the central and apparently oldest neoplasm in this situation, with a histological structure similar to that of the older tumors about it, also may be of implantation origin. Other sections in the series indicate that the carcinoma in the central tumor well may have invaded the fused mucosal folds beneath its constricted base (pedicle). This phase of the picture shown here is very similar to that illustrated in Figure 101 and also to that found in other serosal implantations of carcinoma. A further discussion of the conditions shown in this microphotograph appears in the report of this case (Case 9). × 25.



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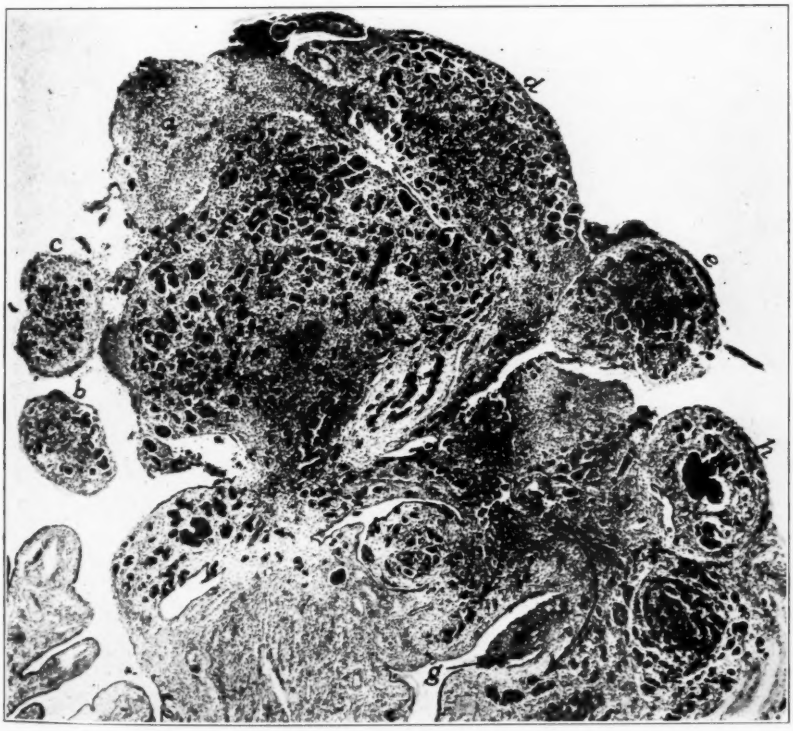

107

Sampson Implantation Carcinoma of Tubal Mucosa

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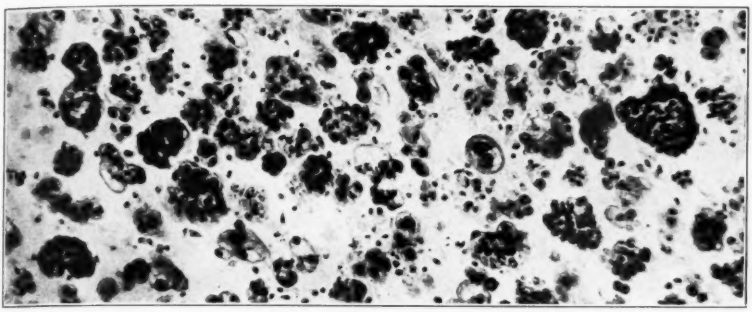
Plate 107

Fig. 108. Section of the sediment from the centrifugalized ascitic fluid in which the fimbriae shown in the preceding microphotographs were immersed. Note the clumps of viable appearing cancer cells, which might become attached to and grow either on the tubal serosa or on the fimbrial mucosa if a suitable soil should arise in these locations (see Figs. 98, 101, 106 and 107).

Fig. 109. A portion of a longitudinal section of the ampulla of the left tube near its abdominal ostium from the same patient as the cancer cells shown in the preceding microphotograph. The lumen of the entire ampulla of this tube contains cancer cells similar to those shown in the preceding microphotograph. The fimbriae of this tube are grossly and histologically normal. It is believed that cancer cells (similar to those shown in Fig. 108) entered the lumen of this tube through its abdominal ostium. Note that in this field the mucosal epithelium is intact and that there is no visible attempt on the part of the cancer cells to replace it. × 130.

Fig. 110. A portion of the same section shown in the preceding microphotograph but nearer the isthmus of the tube. Here a change has taken place. The tubal epithelium not in contact with cancer cells is intact. On the other hand, cancer cells are growing and apparently attaching themselves to another portion of the mucosa by actually replacing the epithelium. × 130.

Fig. 111. A field similar to the preceding one and from the same section. The replacement of mucosal epithelium by carcinoma is even more evident here than in the preceding microphotograph. It has been shown that the mucosa of the ampulla of the tube is abundantly supplied with lymph vessels. Judged lymph vessels are marked "a" and "b." The clump of cancer cells "c" well may be in a lymph vessel. I believe that the carcinoma in this vessel came from the secondary carcinoma of the tubal mucosa rather than from the ovarian tumor by lymphatic embolism or permeation. No evidence of the latter was detected in this tube. Carcinoma was not found in lymph vessels of other portions of this tube. On the other hand, in spite of the examination of several sections of this block, I was unable to demonstrate the invasion of this lymph vessel by the nearby growth of the mucosa. Since complete serial sections had not been made, this phenomenon, though present, could have been missed. × 130.



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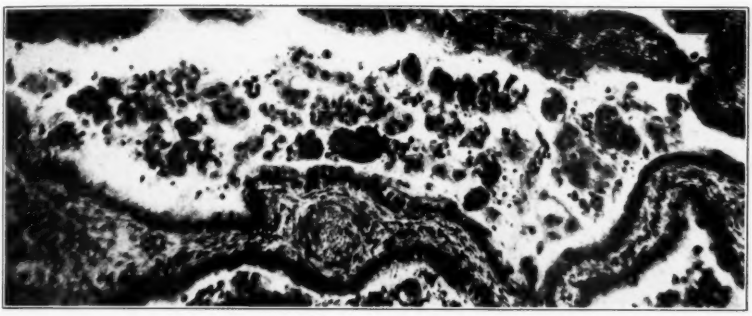

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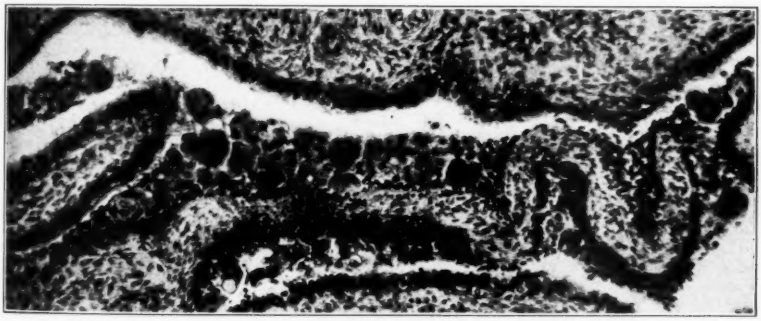

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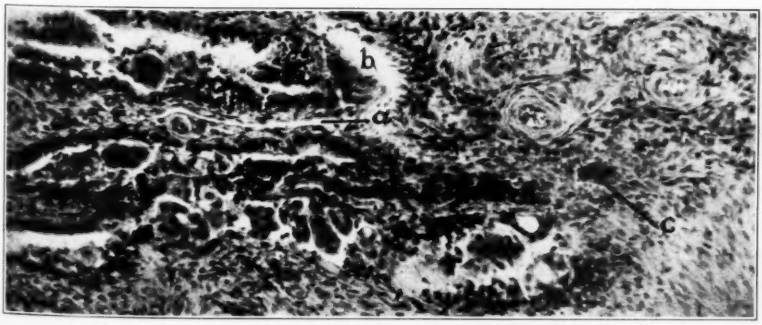

111

Sampson Implantation Carcinoma of Tubal Mucosa

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Plate 108

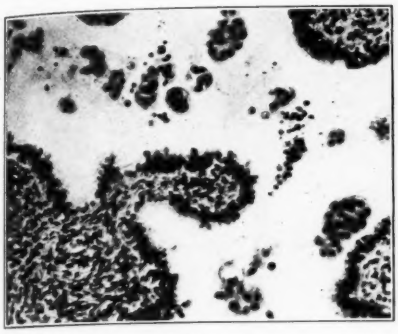
Fig. 112. A portion of a longitudinal section of the ampulla of the right tube, the fimbriated end of which is shown in Figure 102. Cancer cells singly and in clumps, similar to those in the ascitic fluid (see Fig. 108), are present in all portions of the lumen of the ampulla of this tube. However they are not as numerous as those in the lumen of the opposite tube (see Fig. 109). × 130.

Fig. 113. A portion of the ampulla of the right tube near its isthmus, from the same series as the section shown in the preceding microphotograph. Here cancer cells are in contact with and may be actually growing on a portion of the mucosa lacking its epithelium. The etiology of this raw area of the surface of the mucosa is not evident. × 130.

Fig. 114. Another portion of the right tube, nearer the isthmus than that shown in the preceding microphotograph and from the same series. Here cancer cells have replaced the mucosal epithelium and have actually formed a tumor. The two areas with carcinoma replacing the tubal epithelium (shown in the last two microphotographs) are not continuous. The condition shown in the last field may well represent a later stage of that shown in the preceding one. Compare also with Figures 110 and 111 from the opposite tube of the same patient. A similar phenomenon apparently is present in the two tubes. × 130.

Fig. 115. Carcinoma of the tubal mucosa appears in the left half of this field (from the same series as the preceding section). From a study of other sections of this area it was judged that the carcinoma shown here is another portion of the growth pictured in the preceding microphotograph. I do not believe that this portion of the carcinoma is in a lymph vessel. However, carcinoma "a" well may be in a mucosal lymph vessel. × 130.

Fig. 116. A portion of the right tube near that shown in the preceding microphotograph. Carcinoma ("a") is shown in judged lymph vessels, probably two portions of the same vessel. Complete serial sections of this block had not been made. Nevertheless from the sections available I believe that probably the carcinoma shown in the judged lymph vessels ("a" of this and of the preceding microphotograph) may be continuous. Carcinoma was found in the lymph vessels of the mucosa of this tube only in the fimbriae (see Fig. 102) and in the ampulla, near the isthmus, shown here. Carcinoma was found in subserosal lymph vessels beneath some of the larger serosal implants of this tube (see Fig. 101 of this paper, and Figs. 85 and 89 of a previous paper [10]). I fully realize that the carcinoma which has replaced the ampullar epithelium, in Figure 114, may have reached this situation by metastasis from the ovarian carcinoma through the lymph vessels since the carcinoma in the ovary had invaded the lymph vessels of that organ. On the other hand, very strong circumstantial evidence has been presented indicating that cancer cells escaping from the ovarian tumor into the peritoneal cavity entered the lumens of both tubes, became implanted on their mucosa, and subsequently invaded the lymph vessels of the latter (see Figs. 108 to 115 inclusive). × 130.



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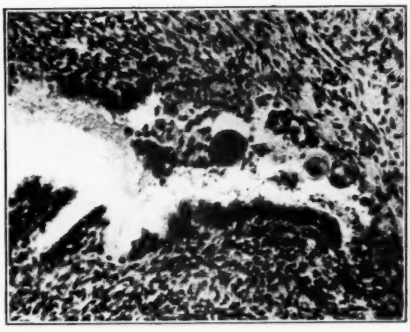

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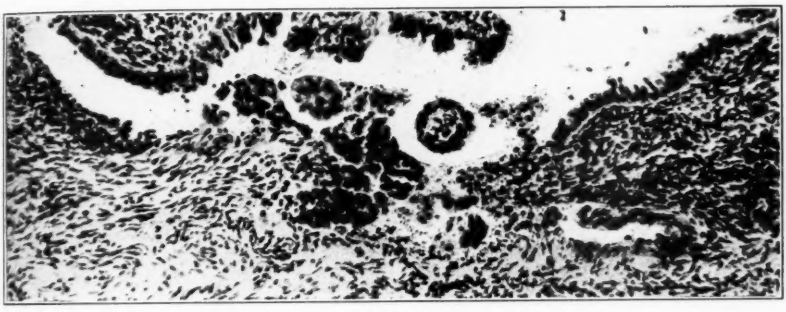

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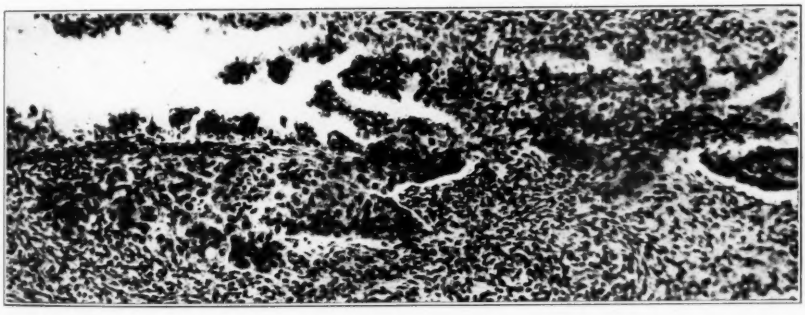

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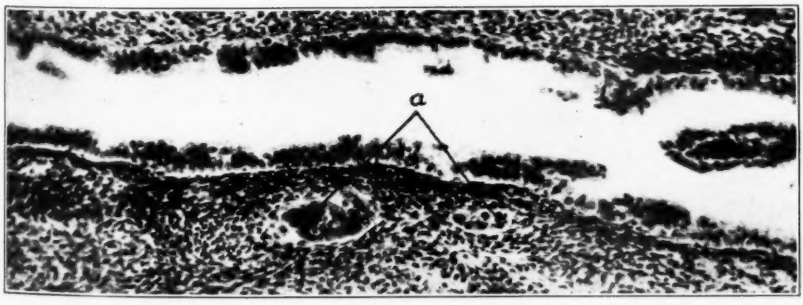

116

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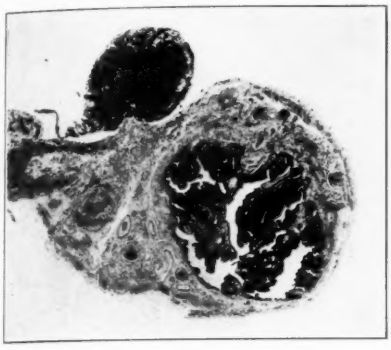
FIG. 117. A polypoid carcinomatous implant on the serosa of the tube from a patient with carcinoma of both ovaries and peritoneal carcinomatosis (Case 4 of a previous paper[9] and Case 10 of the present paper). The implant is attached to the wall of the tube by a short vascular pedicle. × 5.

FIG. 118. Two polypoid implantation-like metastases ("a" and "b") of the mucosa of the opposite tube (Case 10). The larger one is attached to the mucosa by a short vascular pedicle. The pedicle of the smaller and apparently younger implant, situated below the larger one, was missed in the few sections which were made of this tube. The various stages in the development of metastases like these, from cancer cells becoming implanted in granulation tissue arising on the surface of the mucosa of the tube, were not found in this case. However, it is demonstrated in other instances in this article (see Figs. 27, 28, 29, 46, 47, 65 and 68) and may have been missed in this case since the entire tube was not studied microscopically. Clumps of cancer cells are present in the lumens of both tubes but were not found in the lymphatics of either of the tubes or the ovaries. However, they were found in lymph vessels beneath implants on the parietal peritoneum (see Fig. 120 of previous paper[9]), and also in a lymph vessel in the peritoneum of the mesosalpinx beneath the pedicle of the polypoid implant shown in Figure 121 of this paper. × 5.

FIG. 119. A pedunculated polypoid implant on the serosa of the mesosalpinx of the tube shown in Figure 117. It is not only smaller but also apparently younger than the preceding serosal implant. Cancer cells in the peritoneal cavity may become implanted on recently newly formed tissue arising on the surface of such an implant. Carcinoma embedded in the implant may invade its stroma and reach its surface. × 25.

FIG. 120. Higher magnification of the smaller metastasis on the tubal mucosa shown in Figure 118. Compare with the serosal implant shown in the preceding microphotograph. The histological structure of the two tumors is quite similar but not identical. The stroma of this metastasis is more compact and less vascular than that of the preceding one. It resembles more closely the stroma of the polypoid serosal and mucosal implants shown in Figures 122, 124, 128, 131 and 138. A portion of the lower pole of the larger metastasis of Figure 118 appears in the upper portion of this microphotograph. The carcinoma on the surface of the lower pole of this neoplasm may have arisen from the implantation of cancer cells on its surface or from the extension of the carcinoma in the neoplasm. Both of the metastases, shown here, have developed in polypoid newly formed tissue arising on the surface of the mucosa as in serosal implants. Carcinoma was not found in lymph vessels of any portion of the tube but was detected in one situation in the mesosalpinx (see the next microphotograph). I believe that all of the four metastases, just shown, had a similar origin. × 25.

FIG. 121. A polypoid implant on the serosa of the mesosalpinx shown in Figure 119. It is larger and apparently older than the preceding serosal implant. Lymph vessels cannot be detected in its vascular pedicle. However, cancer cells ("a") are present in a judged lymph vessel of the serosa beneath the attachment of the pedicle of the implant. I believe that these cells probably came from the carcinoma in the implant above them rather than from the primary ovarian tumor by retrograde lymphatic metastasis. × 25.



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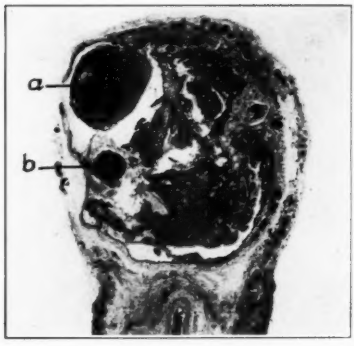

118

119

120

121

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PLATE 110

FIG. 122. Two mature polypoid carcinomatous implants on the serosa of an epiploic appendage from a patient (Case 11) with carcinoma of both ovaries and a peritoneal carcinomatosis. The larger implant is attached to the appendage by a slender vascular pedicle (see the next microphotograph). The smaller implant is pear-shaped and is attached by its small end to the appendage. The carcinoma in the implants had not invaded the preexisting tissues of the appendage. Carcinoma could not be found in the lymph vessels of this appendage. × 10.

FIG. 123. A portion of the pedicle ("a") of the larger implant shown in the preceding microphotograph, at its attachment to the appendage. × 10.

FIG. 124. Three mature polypoid implants on another epiploic appendage from the same patient as the preceding ones. The largest one is sessile: the growth in its base has invaded the appendage at "a." The second implant ("b") is attached to the appendage by a short pedicle. Carcinoma can be seen, under higher magnification, extending through the pedicle toward the appendage. However, the invasion of the appendage by the carcinoma in the pedicle could not be detected. A very small implant ("c") with a slender pedicle is shown, at the right of the second implant. Blood vessels in the appendage accompanied by lymph vessels with carcinoma in their lumens are marked "d." × 5.

FIG. 125. The same implants, shown in the preceding microphotograph, from a section further on in this series. Carcinoma beneath the serosa of the appendage (see Fig. 127) is indicated by "a." This is very near the attachment of the pedicle of the second implant, see the preceding microphotograph. However, I was unable to detect its continuity with the growth in the implant above it. This might have been missed as the series of sections was not quite complete. Carcinoma is present in the serosa of the appendage at "b" and also in lymph vessels accompanying blood vessels (see the dark areas in the appendage above the serosa just mentioned). Sections further on in the series suggest that the carcinoma indicated by "a" may be continuous with that in the lymphatics in the other dark areas. × 5.

FIG. 126. Higher magnification of the blood vessels accompanied by lymph vessels with carcinoma in their lumens, marked "d" in Figure 124. A large lymph vessel with carcinoma in it is marked "a." Smaller vessels also with carcinoma in them are marked "b" and "c." Did the carcinoma in these vessels come primarily from the ovarian tumors through the ovarian lymph vessels and thence, in a roundabout manner, to the lymph vessels of the appendage, or did the carcinoma in the implants on the surface of the appendage invade the underlying lymph vessels? (See Figs. 128, 129 and 130.) × 130.

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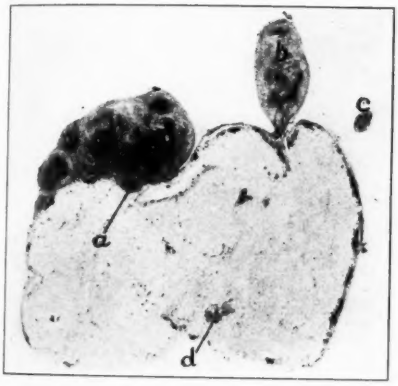

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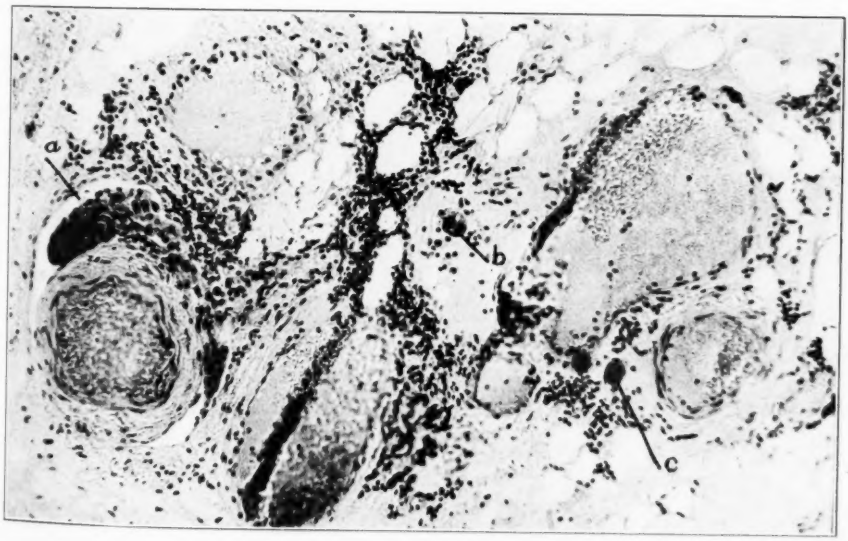

126

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PLATE 111

FIG. 127. Higher magnification of a portion of the second implant and the appendage beneath it shown in Figure 125. The lower portion of the body of the implant appears in the upper half of the microphotograph. The dark area "a" of the tissues of the appendage consists of carcinoma surrounded by dense fibrous tissue. Note that it is below the serosa. Carcinoma was not found either in the serosa or on its surface in this situation. As stated in the legend of Figure 125, the continuity of the carcinoma in this implant with that in the appendage below it was not demonstrated. × 25.

FIG. 128. A large, pear-shaped, sessile polypoid implant and a portion of the appendage beneath it. This implant is attached to the appendage, near the area marked "b" in Figure 125. Note that carcinoma is present in the thickened serosa ("a") of the appendage and, I believe, is continuous with the growth in the implant above (see the next microphotograph). Also compare with the area, marked "a" of the preceding microphotograph, where the carcinoma is below the serosa. × 25.

FIG. 129. The attachment of the implant, shown in the preceding microphotograph, to the epiploic appendage. The implant evidently was injured during the removal of the appendage and is partially torn from the latter. However, a portion of the implant is still attached to the serosa of the appendage in the bottom of an indentation of the appendage into which the base of the implant fitted. The implant is also still attached to the appendage in the upper left-hand portion of the field shown in this microphotograph. The carcinoma of the implant has invaded the epiploic appendage above and to the right of the blood vessel marked "a." × 25.

FIG. 130. A higher magnification of the area about the blood vessel "a" of the preceding microphotograph but from a section further on in this series. A blood vessel, a continuation of vessel "a" of the preceding section, can be followed into the deeper tissues of the appendage. This vessel is accompanied by a lymph vessel distended with carcinoma (see arrows) which evidently came from the growth situated about the upper end of the upper arrow. The carcinoma just mentioned is a continuation of the growth in the base of the implant at its attachment to the appendage shown in the preceding microphotograph. I am convinced the carcinoma in the lymph vessels of the appendage came from this source and not from the ovarian tumor by roundabout and retrograde lymphatic permeation or metastasis. Compare with Figure 122 in which an epiploic appendage had similar implants with no evidence of an extension of the carcinoma from the implants into the appendage and without carcinoma in its lymph vessels. × 54.

127

128

129

130

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Implantation Carcinoma of Tubal Mucosa

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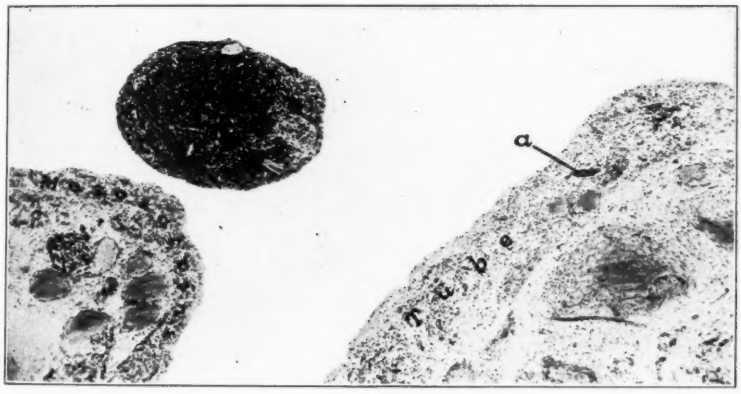
PLATE 112

FIG. 131. A mature pedunculated polypoid implant attached to the mesosalpinx, from the same patient as the preceding ones. The structure of this implant is similar to the one shown in Figure 128. On the other hand there is no evidence of the extension of the growth into the preexisting tissues of its host nor of carcinoma in the lymph vessels of the mesosalpinx beneath it. × 25.

FIG. 132. The small end of a sessile pear-shaped polypoid implant, near its base. This implant resembles in shape the smaller implant shown in Figure 122 and is attached to the right tube near the origin of the mesosalpinx. The structure of this portion of this implant is the same as that of the base of the implant proper shown in the preceding microphotograph. A section through the large end of this pear-shaped implant appears in Figure 144. Carcinoma is present in a subserosal lymph vessel of the tube at "a." What is the relation between the carcinoma in the implant and that in the subserosal tubal lymph vessel? × 25.

FIG. 133. The attachment of the sessile pear-shaped implant shown in the preceding microphotograph to the serosa of the tube. All of the tissue shown in this field except the carcinoma is preexisting. A horizontal section of a ridge of the tubal serosa to which the implant is attached presents under low magnification the appearance of the long slender pedicle of a polypoid implant. The carcinoma in this field is continuous with that shown in the preceding microphotograph. Carcinoma ("a," "b" and "c") is shown in lymph vessels. It can be demonstrated that the carcinoma in the first two vessels is continuous with the growth between them and that carcinoma "c" is a continuation of carcinoma "a." Note carcinoma is not present in the lymphatics of the mesosalpinx beneath the attachment of the implant shown in Figure 131 and that the growth in the implant has not invaded the mesosalpinx. See also similar implants on an epiploic appendage (Fig. 122) without carcinoma in the lymph vessels of the appendage. The conditions just demonstrated in the present implant are analogous to those shown in implants on the epiploic appendages of Figures 124 to 130 inclusive. I believe that the carcinoma in the lymph vessels of the tube, as in those of the last mentioned epiploic appendage, primarily came from the nearby implant and not from the ovarian tumor by retrograde lymphatic metastasis or permeation. × 25.

131



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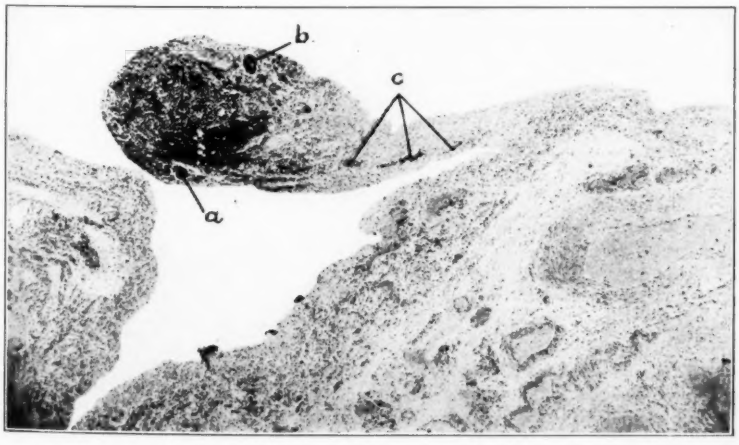

133

Sampson Implantation Carcinoma of Tubal Mucosa

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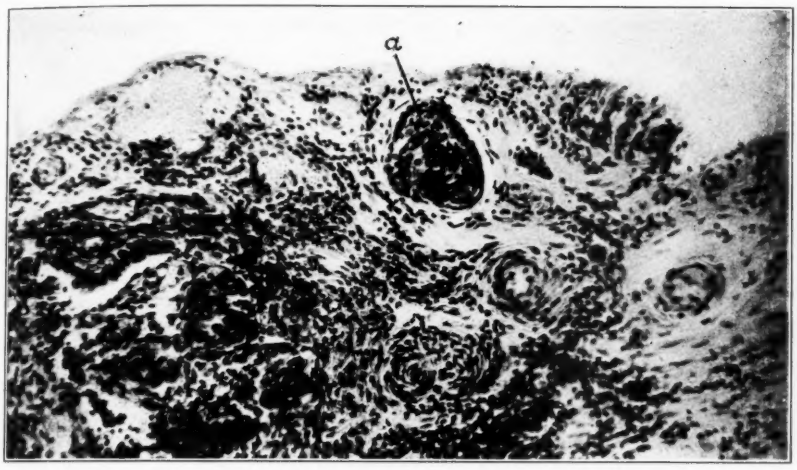
Plate 113

Fig. 134. Higher magnification of a portion of the serosa and subserosa of the tube shown in the preceding microphotograph, from the same section. The tissues of the serosa, in the left-hand portion of this field, have been invaded by carcinoma from the implant which is attached to the tube in this situation. A lymph vessel is distended with carcinoma ("a"). The latter can be shown in the series of sections to be a continuation of the nearby carcinoma below and at its left. × 130.

Fig. 135. Higher magnification of a portion of the pedicle-like structure shown in Figure 133. This structure is not newly formed but is a ridge of the tubal serosa which has been cut horizontally. Carcinoma in a lymph vessel is spreading either from or toward the implant. I believe the former. × 130.

Fig. 136. Higher magnification of the tubal serosa and subserosa in which the carcinoma in a lymph vessel, indicated by "a" of Figure 132, is situated. Another lymph vessel or a portion of the one above is marked "b." The primary source of the carcinoma in lymph vessel "a," whether from the invasion of the ovarian lymph vessel by the ovarian tumor and an upstream metastasis from this source, or from a downstream spread of carcinoma implanted on the serosa of the tube with subsequent invasion of the underlying lymph vessels, is suggested in the two preceding microphotographs. × 130.



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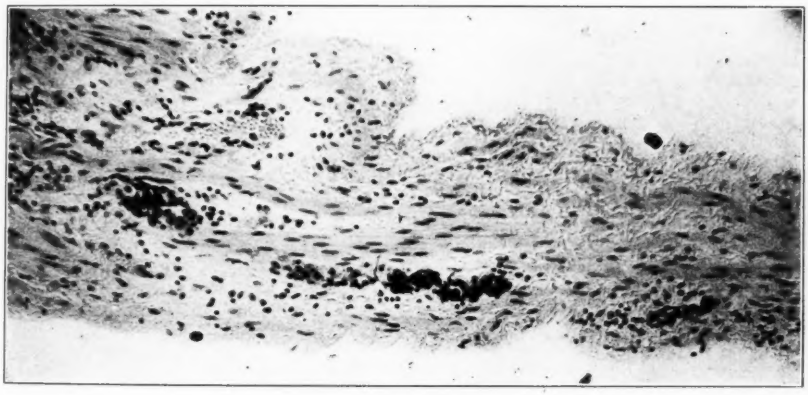

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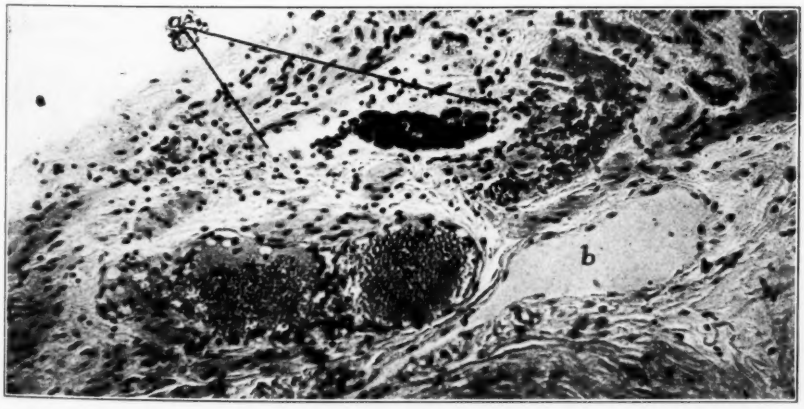

136

Sampson

Implantation Carcinoma of Tubal Mucosa

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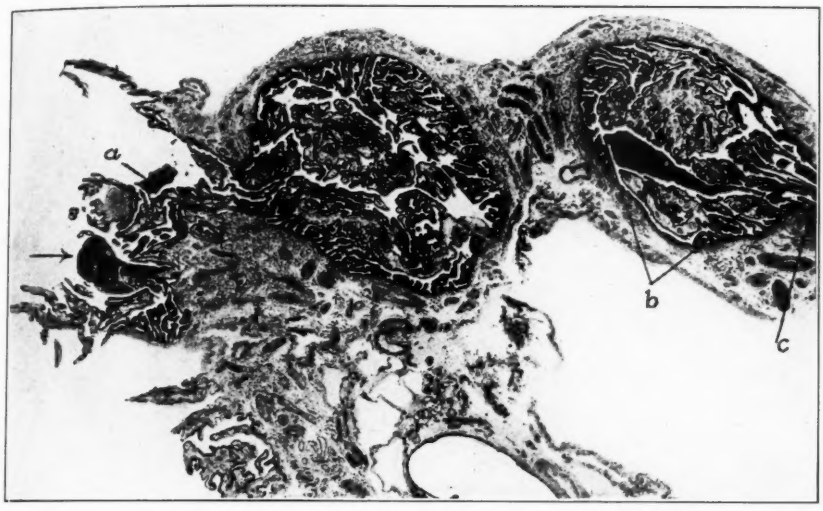
FIG. 137. Longitudinal section of the distal end of the right tube including a portion of the mesosalpinx and the ovarian fimbriae along its free margin (Case 11). Carcinoma appears in this section in four situations. The most important one is a metastasis to a mucosal fold of the fimbriae marked by the arrow. The other three consist of various sized collections of cancer cells ("a," "b" and "c") occurring singly and in clumps. The cancer cells are held together by blood-stained débris which includes unidentified cells. These collections of cancer cells and the material about them are identical with those floating about in the ascitic fluid of this patient (see Fig. 140). They resemble rafts in the section. In this section a small raft ("a") appears just outside the abdominal ostium of the tube. A larger one ("b") is situated in the ampulla and still another but smaller one in the ampulla is marked "c." In other sections of this series they appear in all portions of the ampulla of the tube. In other sections a large raft can be seen in the lumen of the abdominal ostium, half of it lying between the fimbrial folds and the other half within the ampulla. Evidently these cells came from the abdominal cavity and have migrated into the lumen of the tube through its abdominal ostium. × 5.

FIG. 138. A higher magnification of the metastasis to the mucosal fold of the fimbriae marked by the arrow in the preceding microphotograph. A polypoid growth of newly formed tissue, with carcinoma embedded in it, is attached to a mucosal fold by a short slender pedicle ("a"). The histological structure of this polypoid growth is similar to that of many pedunculated, polypoid serosal implants (compare with Fig. 131 from the same patient). An outgrowth of very early granulation tissue ("b") with cancer cells embedded in various portions of it (see Fig. 142) has developed on the under surface of the polypoid outgrowth. This is one way that implants on both the serosa and the mucosa may increase in size (see Figs. 101 and 107). Carcinoma is present, to the right, in the mucosal fold from which the newly formed stroma of the judged implant arose and is apparently continuous with that in the newly formed tissue (see also the next microphotograph). Carcinoma was not found in any of the lymph vessels of the mesosalpinx or wall of the tube in this situation. However, it is present in lymph vessels of this mucosal fold (see "c") and in those of a few nearby folds. I realize that this metastasis may possibly have arisen from the ovarian tumor by an upstream spread through the lymph vessels. However, I believe that the implantation of cancer cells in granulation tissue arising on the fimbrial fold with subsequent invasion of the fold and a downstream spread of the tumor in the lymph vessels of the mucosa is a more likely explanation of the condition just described (see Figs. 129, 130 and 133 from the same patient). × 25.

FIG. 139. Higher magnification of a portion of both the implant shown in the preceding microphotograph and the mucosal fold to which it is attached. The implant appears at the left and the mucosal fold at the right with the pedicle or bridge between the two. The continuity of the carcinoma in the two situations is very strongly suggested but cannot be positively demonstrated. Note that the stroma of the pedicle appears to be constricted. × 130.



137

138

139

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Implantation Carcinoma of Tubal Mucosa

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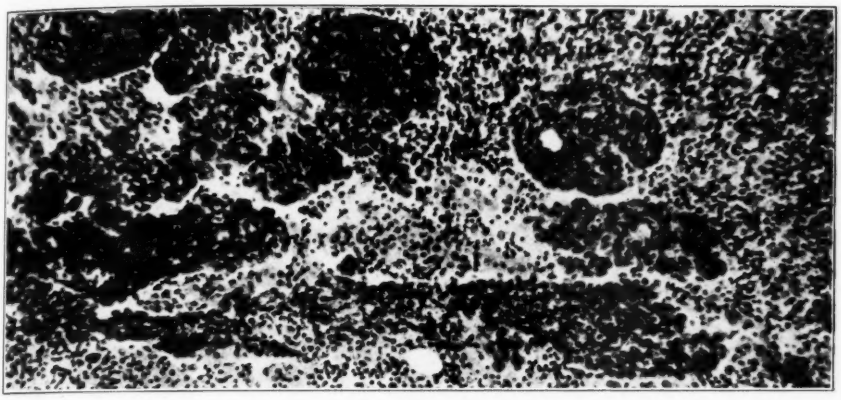
Plate 115

Fig. 140. Section of the sediment obtained by centrifugalizing some of the ascitic fluid from Case 11. Cancer cells singly and in clumps are present in this sediment. While some of the cancer cells appear to be dead, others stain well and appear to be alive and possibly to have increased in numbers. The fimbriae of the tube shown in Figure 137 were immersed in the ascitic fluid. × 130.

Fig. 141. Clumps of healthy appearing cancer cells in the collection of cells marked "a" between the fimbrial folds shown in Figure 137. Cells like these in contact with the surface of the mucosal folds might become implanted on them when a suitable soil is created for this phenomenon (see the next microphotograph). × 130.

Fig. 142. Higher magnification of a portion of the mass of early granulation tissue arising from the under surface of the judged polypoid implant on the mucosal fold shown in Figure 138. Cancer cells similar to those shown in the preceding microphotograph are embedded in this tissue. The larger mass of cancer cells marked "a" is not completely surrounded by this tissue at its right. The small clump of cancer cells ("b") is embedded in the tissue as are also those (a little out of focus) marked "c." The pedunculated implant shown in Figure 138 is increasing in size by the development of granulation tissue, on its surface, in which cancer cells are becoming embedded. In like manner this implant may have arisen. Note that the cancer cells in the lymph vessel ("d") of the mucosal fold, have grown into a mass similar to the clumps of cancer cells in the ascitic fluid shown in Figure 140. The carcinoma in the mucosal fold shown in Figure 138 could easily have invaded the lymph vessels of that fold. × 130.

Fig. 143. Higher magnification of a portion of the smaller collection of cancer cells in the lumen of the ampulla of the tube indicated by "c" in Figure 137. I believe that these cells migrated from the abdominal cavity into the tube through its abdominal ostium. Compare with Figures 140 and 141 and note that the cancer cells appear to be alive. It is evident that the mucosa of the ampulla is exposed to these cells in the same manner as the peritoneum and the fimbrial mucosa. If a suitable soil should arise in the ampullar mucosa we would expect that these cells might become implanted on this mucosa as I believe they become implanted on the serosa and also on the fimbrial mucosa. × 130.



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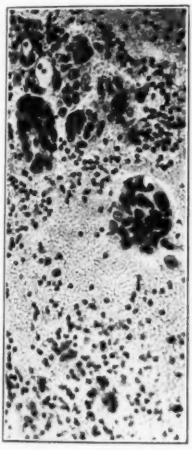

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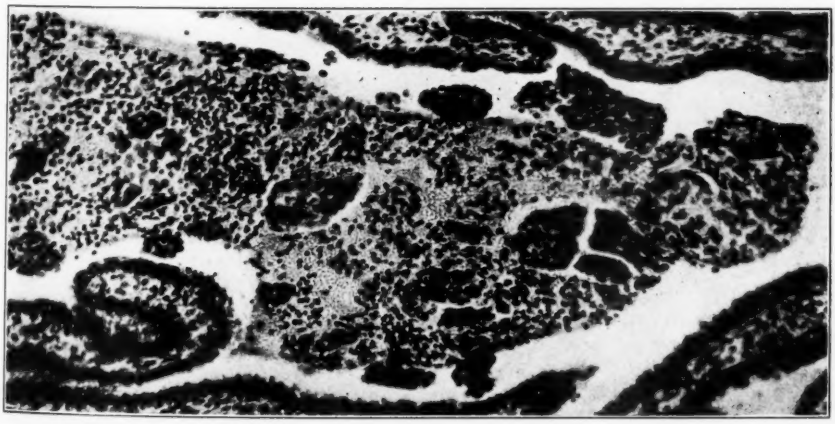

143

Sampson

Implantation Carcinoma of Tubal Mucosa

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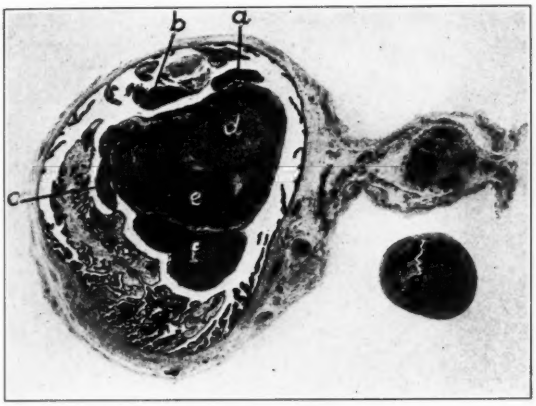
FIG. 144. Cross section of the mid-portion of the ampulla of the tube shown in Figure 137. A serosal implant ("g"), similar to other serosal implants from the same patient (see Figs. 122, 123, 124 and 131), appears below the mesosalpinx. This implant is pear-shaped and is attached by its small end to the tube near the mesosalpinx (see Fig. 133). The tube is distended by an irregularly shaped conglomerate tumor. In the lumen of the tube above this tumor appear collections of cancer cells ("a" and "b") similar to those shown in Figures 137 and 143. A collection of cancer cells ("c") similar to "a" and "b" is partially fused with the tumor and is an indication not only of the way the tumor is now increasing in size, but also of the way the tumor may have grown. The nucleus ("d") of the tumor appears in the upper right-hand portion. This nucleus consists of a partially encapsulated rounded mass of vascular, newly formed connective tissue with carcinoma embedded in it and also of clumps of cancer cells, in various stages of implantation, on portions of its surface and thus exposed to the lumen of the tube. The nucleus is partially necrotic in its upper right-hand portion. This necrosis involves both the stroma and the carcinoma in it. The histological structure of the nucleus is similar to that of the serosal pedunculated polypoid implants (Figs. 122, 124, 128 and 131) and of the one pictured in this microphotograph, and also of the implant on the mucosa of the fimbriae shown in Figure 138, all from the same patient and all of judged implantation origin. The stroma of the nucleus differs from that of the implants just mentioned in that it contains more blood vessels and its connective tissue is more fibroblastic. Fused with the lower half of the nucleus is a large, deeply stained irregular shaped mass ("e") which for the most part is necrotic. The shadows of clumps of cancer cells in various stages of dissolution can be detected in the stroma of this mass. The mass just described and the nucleus have a common capsule with scattered clumps of viable appearing cancer cells embedded in portions of the capsule covering mass "e." Fused with the lower surface of this capsule is a pear-shaped mass ("f") smaller and paler than the mass ("e") above it. It is not as necrotic as the former and consists mainly of cells indistinguishable from the nonmalignant undiagnosed cells about the cancer cells in the ascitic fluid shown in Figure 140, and about the collections of cancer cells in other portions of the ampulla shown in Figure 143. A few clumps of recognizable cancer cells and more and larger clumps of judged necrotic cancer cells are present in this mass. Fibroblasts, connective tissue and blood vessels cannot be detected in masses "e" and "f." It is clearly evident, from the study of this and other sections, that this conglomerate tumor has grown from the implantation of clumps of cancer cells, singly and collectively, on the surface of a nucleus of newly formed tissue which has the structure of a polypoid implant. It is also evident that the blood supply of this nucleus is inadequate, not only for the maintenance of the more recent attempted implantations but for the life of the nucleus itself. Hence the necrosis in these situations. × 5.

FIG. 145. Higher magnification of a portion of the tumor, with a cross section of its pedicle ("a") and the ampullar mucosa beneath the pedicle, shown in the preceding microphotograph. This field is from a section at some distance in the series from the preceding one. In this section the tumor appeared much smaller than in Figure 144. There is an attempted implantation of clumps of cancer cells on its under surface. × 54.

FIG. 146. A field similar to the preceding one and quite near it in the series of sections. The origin of pedicle "a" from a mucosal fold is shown. Blood vessels do not appear in this section of the pedicle but are evident in a nearby section which, on the other hand, does not show the origin of the pedicle or the carcinoma "b" and "c" in the mucosal lymph vessels as well as this section does (series of sections not quite complete). A further discussion of the conditions shown in these last two microphotographs appears in the report of this case (Case 11). × 54.



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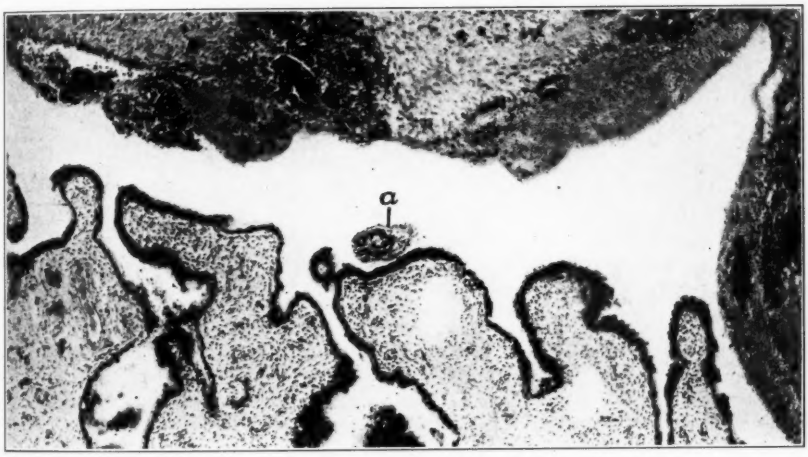

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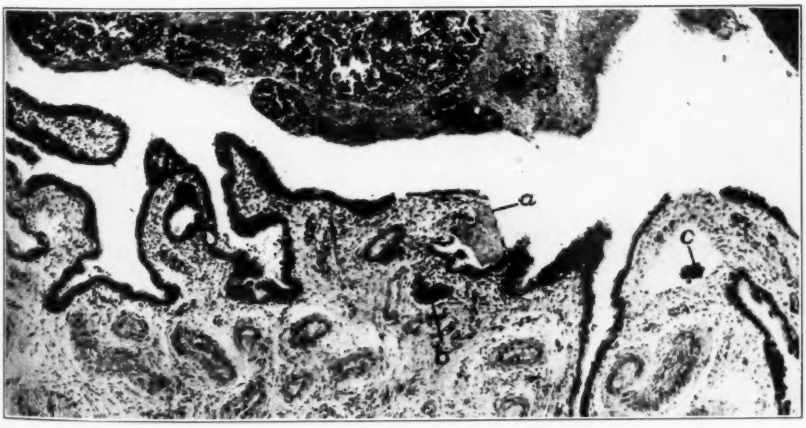

146

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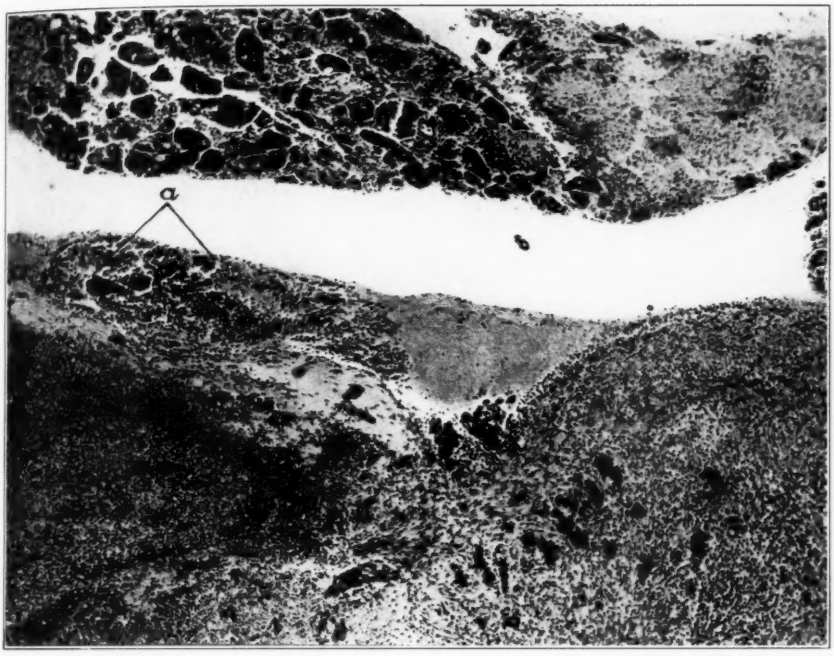
FIG. 147. Higher magnification of a portion of the mucosal tumor (implant) and of the large collection ("b") of clumps of cancer cells above it (shown in Fig. 144). I believe that the clumps of cancer cells migrated from the peritoneal cavity through the abdominal ostium of the tube (see Fig. 137). The well organized vascular nucleus of the tumor is shown at the right with viable appearing carcinoma in it and on its surface. A portion of the necrotic mass "e" of Figure 144, which, I believe, represents the unsuccessful implantation of a large collection of clumps of cancer cells, similar to the one shown above the tumor, appears at the left. On the surface of the capsule of this necrotic portion of the tumor is an attempted implantation of cancer cells ("a"). × 54.

FIG. 148. A clump of cancer cells in contact with the intact epithelium of the ampullar mucosa of the tube shown in the preceding section. There are many such clumps of various sizes in all portions of the lumen of this tube. Since the cells stain as well as the tubal epithelium it would seem that they might live a long time in the lumen of the tube as similar cells live in ascitic fluid and in the lumens of lymph vessels. These clumps of cells vary greatly in size and therefore may multiply in this situation even though mitotic figures are not evident. The latter were not evident in the primary ovarian tumor, in the growth in the lymph vessels or in the cancer cells in the ascitic fluid. Note the deeply staining cell in the normal appearing tubal epithelium beneath the clump of cancer cells. I wish I knew its significance. There is no appreciable local reaction on the part of the subepithelial tissues of the mucosal fold. × 130.

FIG. 149. A field similar to the preceding one and from the same section. A small clump ("a") of detached cancer cells appears in the lumen of the tube. Above and to the right of this is a larger clump of cancer cells which is attached to the surface of the mucosa. There is a marked reaction, indicated by lymphocytes, in the subepithelial tissue of the mucosa beneath the attachment of the cancer cells. Lymphocytes have not only grown out between the mucosal epithelial cells but have actually replaced some of the latter. They also have extended into the clump of cancer cells. The phenomenon just described, the only one of its kind encountered in this series of sections, may well represent an early stage in one type of implantation of cancer cells on tubal mucosa. × 130.

FIG. 150. Carcinoma of the mucosa of the ampulla of the tube from the same series of sections as the ones shown in the preceding microphotographs. Only one other tumor like this one was encountered in this series. I do not believe that they are primary carcinomas of the tubal mucosa. They more likely arose from the implantation and subsequent growth of cancer cells which had been floating about in the lumen of the tube (there are many such cells in this tube). The tumor shown here well may represent a later stage of the process shown in the preceding microphotograph. The reaction of the mucosal tissues beneath the attachment of the carcinoma is similar to that shown in the preceding microphotograph. Note that lymph vessel "a" is in close proximity to the growth. If the patient had not been operated upon at this time, but later, the carcinoma, in the meantime, might have invaded the nearby lymph vessel, and its pathogenesis might then have been attributed to metastasis from the ovarian growth through the lymph vessels rather than to implantation. × 130.



147

148

149

150

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Implantation Carcinoma of Tubal Mucosa

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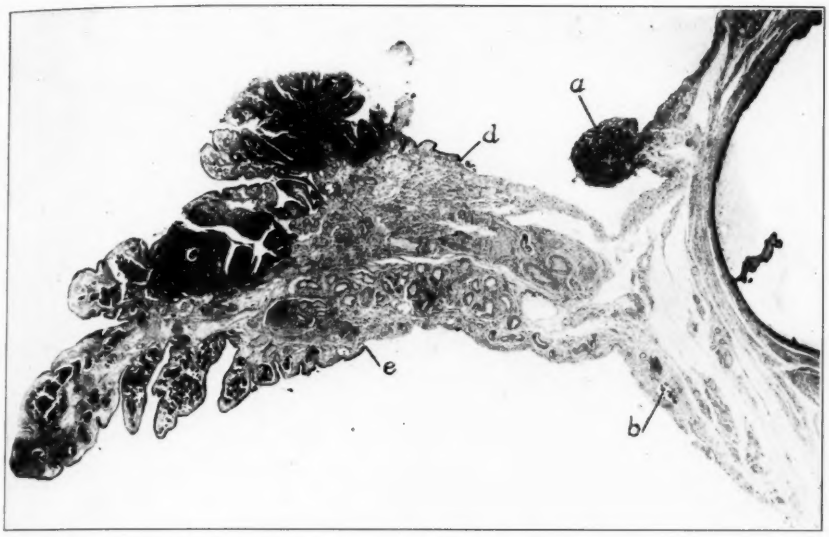
Plate 118

Fig. 151. The free margin of the right mesosalpinx near the tube, including the fimbriae and a portion of the ampulla (hydrosalpinx), from a patient (Case 12) with a large papillary adenocarcinoma of the right ovary (judged secondary carcinoma of the left ovary and tube) and peritoneal carcinomatosis. A portion of the dilated tube appears at the right with a polypoid implant ("a") attached to its serosa. Cancer cells are present in a space ("b") lined by mesothelium. The lymph vessels of the fimbrial mucosa are filled with carcinoma as with an injection mass. In two situations, however, the carcinoma has extended beyond these vessels and invaded the tissues of the folds. Carcinoma cannot be detected in the deeper lymph vessels of the mesosalpinx proper but is shown in lymph vessels below the mucosal fold ("c") which is infiltrated by the growth. Two mucoserosal junctions are indicated by "d" and "e." Note that there is no indication of an anastomosis between the mucosal and serosal lymph vessels in these situations. The portal of entry of the carcinoma into the mucosal lymph vessels of the fimbriae was not detected, owing to an incomplete study of this specimen. The histological picture of the fimbrial folds is one resulting from lymphatic permeation, probably in this instance from the ovarian carcinoma. Implants were not detected in these fimbriae (compare with the fimbriae of the opposite tube shown in Fig. 154). × 10.

Fig. 152. Cross section of the left ovary including a portion of the broad ligament at the right (Case 12). The right lower portion of this ovary has been invaded by carcinoma which well may have come from a growth arising on the surface of the ovary. This phenomenon is more evident in sections (though badly torn) from another block. A large papillary carcinoma appears on the upper left-hand surface of the ovary with encapsulated carcinoma below it. A pedunculated polypoid implant is marked "a." Two sessile polypoid implants ("b" and "c") appear on the serosa of the broad ligament. Portions of two sessile polypoid implants on the under surface of the ovary are marked "d" and "e." The encapsulated carcinoma "f" also well may be of implantation origin. The carcinoma to the left of this presents the appearance of having sunk into the ovary from its surface: the ovarian tissue has not completely encapsulated it (see arrow). The picture shown here is similar to that sometimes found in early ovarian endometriosis. I believe that the carcinoma of this ovary arose from the implantation of cancer cells on its surface, which had escaped from the large carcinoma of the opposite ovary into the peritoneal cavity. Carcinoma is present in a lymph vessel or vessels of the broad ligament, marked "g." Carcinoma was not found in the lymphatics of the broad ligament in the sections of the other block. I believe that the carcinoma in the lymph vessels, just described, probably came from the nearby ovarian tumor rather than primarily from the growth in the opposite ovary by a roundabout, retrograde lymphatic metastasis. × 5.



151

152

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Implantation Carcinoma of Tubal Mucosa

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FIG. 153. A large sessile polypoid serosal implant in the bottom of the anterior cul-de-sac at the junction of the anterior uterine wall with the vesico-uterine reflection of peritoneum (Case 12). The carcinoma in this implant has invaded both the uterine wall and the reflection of peritoneum. Carcinoma is present in lymph vessels of both the uterus and the peritoneum with positive proof, in the latter situation, that it is continuous with the carcinoma in the implant. A pedunculated polypoid implant ("c") similar to "a" of the preceding microphotograph is shown, to the right, on the vesico-uterine reflection of peritoneum. The entire picture, shown here, is one resulting from the implantation of cancer cells on the peritoneum with subsequent invasion of their host and extension into the lymph vessels. × 10.

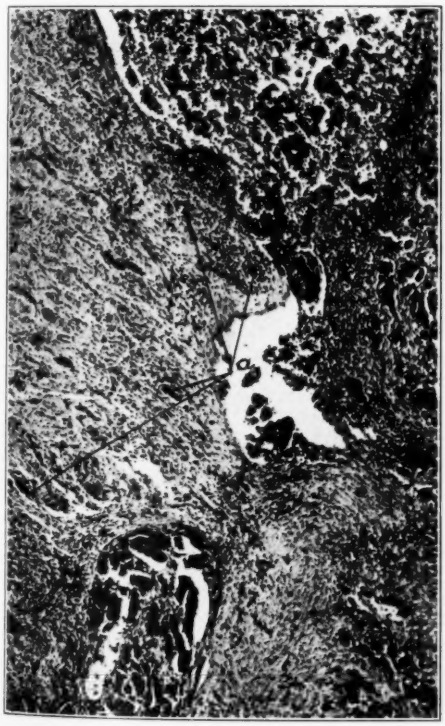
FIG. 154. Longitudinal section of the distal end of the left tube including its fimbriae (Case 12). The carcinoma of the greater portion of the fimbrial mucosa is too far advanced to ascertain either the initial site of the secondary carcinoma of this mucosa or its pathogenesis. Many of the mucosal folds about the ostium of the tube are either partially or completely replaced by the tumor. The growth has spread over the surface of the fimbrial mucosa by replacing its epithelium and also has invaded its lymph vessels. The carcinoma has spread into the ampulla by replacing its mucosal epithelium like a primary tubal carcinoma. The growth has invaded the wall of tube about its ostium, at the right, penetrated the lymph vessels and, here, presents a picture similar to the invasion of the carcinoma in the preceding microphotograph. A pedunculated polypoid serosal implant similar to the one shown in Figure 153 is marked "a." Recent newly formed tissues which have arisen from the fimbrial mucosa, with cancer cells partially and completely embedded in them, are marked "b" and "c." A polypoid implant attached by its slender pedicle to a mucosal fold, similar to serosal implant "a," is marked "d." The area marked "e" presents two phenomena: one, apparently newly formed tissue with cancer cells growing in it, and the other, the invasion of the stroma of the mucosa including its lymph vessels by carcinoma. In many ways this area resembles the sessile polypoid serosal implant shown in the preceding section. We have demonstrated that carcinoma recently has become implanted in newly formed tissue arising on fimbrial mucosa of this tube. This suggests, but does not prove, that the initial secondary carcinoma in this situation also may be of implantation origin with a life history similar to that shown in the preceding illustration. × 8.

FIG. 155. Higher magnification of a portion of the uterine wall beneath the sessile implant shown in Figure 153. Carcinoma in small lymph vessels is indicated by the arms of pointed "a." I believe that the space containing carcinoma, in the lower portion of the microphotograph, is a large subserosal lymph vessel. × 54.

FIG. 156. Higher magnification of a portion of the carcinoma about the ostium of the tube shown in Figure 154. It represents an older portion of the secondary carcinoma but not necessarily its initial site. It presents the histological picture of a primary tubal carcinoma. × 54.

153

154



155

156

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Implantation Carcinoma of Tubal Mucosa

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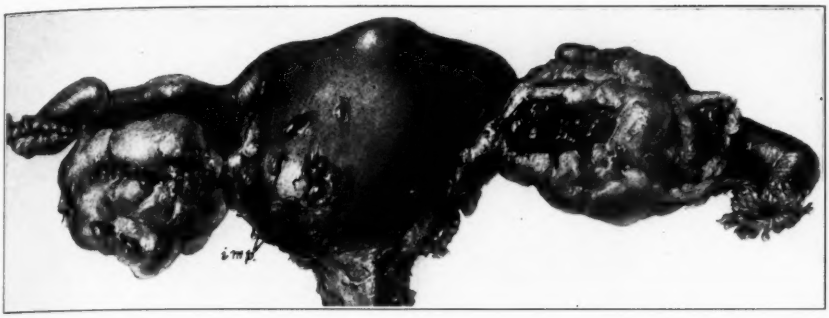
Plate 120

Fig. 157. Posterior view of the uterus, tubes and ovaries from a patient (Case 13) with carcinoma of both ovaries and peritoneal carcinomatosis. The fimbriae of the right tube appear normal. Those of the left tube are thickened and retracted. The carcinoma of the right ovary is more advanced than that of the left, best seen in sections. The uterus contains multiple small myomas and a large mucosal polyp. Implants are indicated on the posterior surface of the uterus and also were present on many other pelvic structures. Natural size.

Fig. 158. An implant on the posterior surface of the uterus shown in the preceding illustration. Apparently carcinoma in the implant has invaded the uterine wall beneath it and reached some of the lymph vessels in this situation. For earlier stages in the life history of implants, similar to this one and from the same patient, see Figure 23 of previous paper [9] and also the next microphotograph. × 25.

Fig. 159. An early implantation of carcinoma on the mesoappendix, from the same patient as the preceding implant. Carcinoma does not invade the mesoappendix in this situation and cannot be detected in the lymph vessels beneath the implant. × 25.

Fig. 160. An implant on the surface of the appendix near the attachment of the mesoappendix, at the left, from the same section shown in the preceding microphotograph. This implant is an old one: a portion of the original tumor has become necrotic and sloughed away causing the ulcer shown here. The carcinoma in the implant has invaded the wall of the appendix and has also extended between the appendix and the mesoappendix, at the attachment of the latter to the appendix. Carcinoma is present in the lymph vessels of the appendix beneath the implant and is judged to have come from the growth implanted on its surface rather than primarily from the ovarian tumor by a roundabout and retrograde lymphatic metastasis. For earlier stages in the life history of implants, similar to this one and from the same patient, see Figure 24 of previous paper [9] and also the two preceding microphotographs. × 25.



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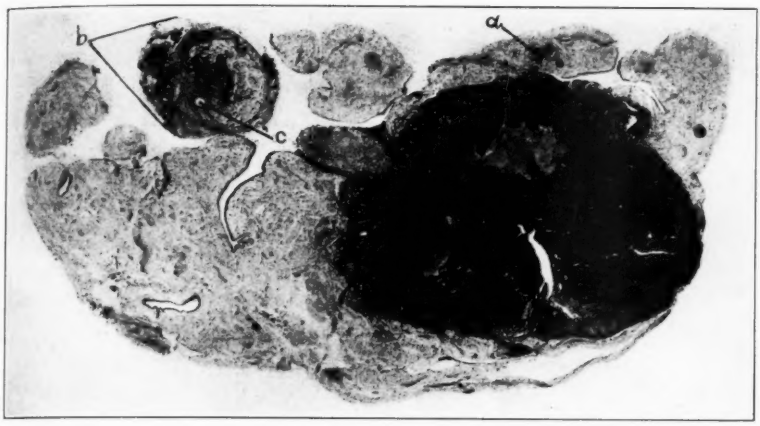
160

UNIV. OF MICH.

FIG. 161. Cross section of the fimbriated portion of the left tube shown in Figure 157. This section is through the largest portion of the secondary tumor and therefore judged to be possibly the oldest. The growth is so far advanced that it is impossible to determine either the initial site of its origin or its pathogenesis. A continuous extension from the primary ovarian tumor was excluded. Therefore it must be metastatic. The carcinoma here has invaded and destroyed many of the mucosal folds. It can be seen in the lymph vessel ("a") of one mucosal fold. An implant on the surface of a thickened mucosal fold is marked "b." In the center of this fold is a small amount of carcinoma. An inclusion of tubal epithelium is marked "c." × 10.

FIG. 162. Higher magnification of a portion of the mucosal fold shown in the preceding illustration with an implant, marked "b," on its surface. Newly formed tissue has arisen on this fold showing both a very early stage in the implantation of cancer cells on its surface, at the extreme right, and also later stages in the encapsulation and growth of these cells in the rest of this tissue. The histological picture of this field is similar to that of carcinoma implanted on the serosa (see Figs. 158 and 159). Other sections in this incomplete series suggest that the carcinoma found in the deeper tissues of this fold may be continuous with carcinoma on its surface. Since carcinoma implanted on the serosa may invade the underlying tissues of its host, including the lymph vessels (see Figs. 153, 158 and 160), carcinoma implanted on the fimbrial mucosa should do the same. × 54.

FIG. 163. Cross section of the tube pictured in Figure 161 and from the same series of sections. If the condition shown in Figure 161 indicates the oldest portion of this secondary tumor, that shown here should represent the further extension of the growth in the mucosal folds of the ampulla. Carcinoma is present in lymph vessels "a" and "b." The carcinoma in lymph vessel "a" can be demonstrated in other sections to be continuous with the growth in the mucosa above it. The condition just shown represents the results either of the implantation of cancer cells on the fimbrial mucosa with subsequent extension of the growth and a downstream invasion of the lymph vessels or an upstream metastasis through the lymph vessels from the ovarian carcinoma. The mucosal fold with an implant on its surface, marked "b" in Figure 161, also is present in this section. Compare the conditions shown in the last three microphotographs with those pictured in Figures 158, 159 and 160. Since cancer cells become implanted on the tubal mucosa in the same way (see Fig. 162) that they become implanted on the serosa and since the metastases shown in these six microphotographs have so much in common, it is possible that their pathogenesis and life history might be the same. × 10.



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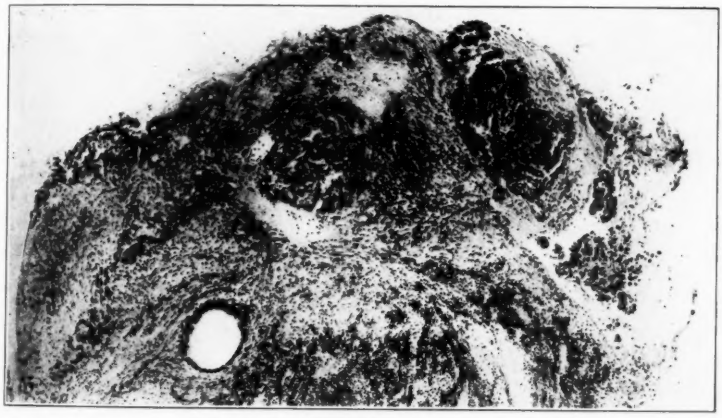

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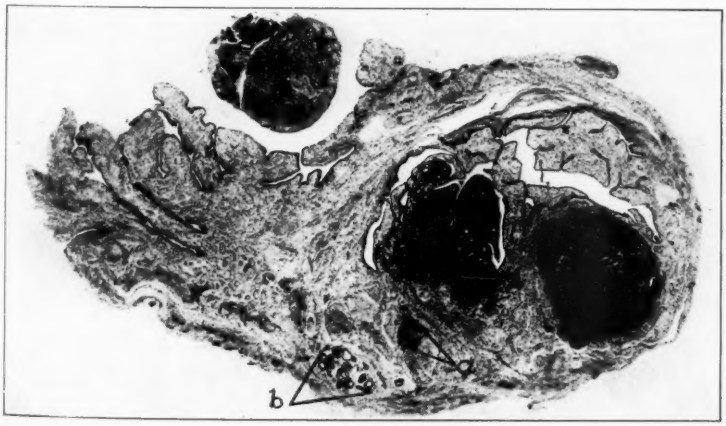

163

Sampson Implantation Carcinoma of Tubal Mucosa

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THE BEHAVIOR OF MURINE AND HUMAN LEPROSY IN FOREIGN HOSTS *

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Efforts to transmit either human or murine leprosy to other species of mammals result frequently in the development of local lesions that ordinarily heal without establishing a progressive disease. The injection of material from such lesions into a second series of animals usually, though not invariably, fails to produce any significant reaction. The inoculum for the first passage often contains lepra bacilli in enormous numbers and even though the infectious material is sterilized in the autoclave, acid-fast bacilli, quite normal in appearance, may be demonstrated for a period of many weeks after injection.[1] The suggestion is commonly offered that the lesion developing at the site of inoculation involves no multiplication of the bacilli but resembles the reaction that occcurs in tissues in the presence of a foreign body. However, there is considerable support for the less conservative interpretation that lepra bacilli may multiply in a foreign host and produce an abortive infection without the usual progressive disease accompanied by extensive metastases. Neither human leprosy nor the analogous disease murine leprosy have been propagated in continuous serial passage in animals of other species.

MURINE LEPROSY

The abortive lesions resulting from the inoculation of human lepromas into lower animals fall short of affording a satisfactory method for experimental study. However, there occurs in nature a form of leprosy in rats which was first described by Stefansky [2] in 1903. Opinions diverge widely concerning the exact relationship of *Bacillus leprae* and *B. leprae murium*; Walker and Sweeney [3] are inclined to regard them as identical, whereas Muir [4] concludes that they are specifically or even generically distinct. The causative microorganisms and the histopathology of human and murine

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leprosy are remarkably similar in their essential features. The attempts to infect volunteers by the inoculation of human leprous tissue have resulted usually in failure; murine leprosy is readily transmissible by ordinary routes of injection from rat to rat. Our efforts have been directed toward securing well established progressive infections of rat leprosy in supposedly refractory species, the ultimate purpose of these observations being to secure some guide for the study of human leprosy in lower animals.

Much of the recent work in this country on rat leprosy has been carried out with the strain that has been propagated in white rats for many years by Dr. E. L. Walker of the University of California. Within a few months after subcutaneous injection local and metastatic lesions rich in lepra bacilli develop with regularity, a result that is in contrast with the less certain behavior of some of the freshly isolated strains.[5] We are grateful to Dr. Walker for his courtesy in supplying us on several occasions with rats showing well established lesions of leprosy and in excellent condition for immediate use. The infectious material was suspended in testicular extract in view of the enhancing effect of such extracts in various types of infection. In particular, Walker and Hoffman [6] have recorded the early and extensive development of lesions in animals inoculated with *B. tuberculosis* suspended in testicular extract. A preliminary test was made to see whether a similar effect might occur in the case of murine leprosy. The testes of a guinea pig were ground with a little sand and suspended in 10 cc. of physiological saline solution. A portion of this was mixed with an equal volume of saline suspension of murine leprosy bacilli and injections of this mixture were made subcutaneously into 4 white rats. Some of the suspension of lepra bacilli was diluted with an equal volume of physiological saline and injected into 4 control rats. Observations of this group of animals at various intervals left an impression that the use of testicular extract enhanced the development of both the local and the metastatic lesions. The effect, however, was not especially striking and eventually physiological saline only was used in preparing suspensions of lepra bacilli.

Experimental Observations

Animals: In addition to white rats we have followed the behavior of rat leprosy in other rodents, namely mice, rabbits and

guinea pigs. Young adult white mice of mixed breeds were used as supplied by commercial dealers. Subsequently some tests were made of the susceptibility of a few inbred strains of mice. Monkeys (*Macacus rhesus*), were also used in order to compare their susceptibility to murine and to human leprosy.

Routes of Inoculation: Especial attention has been given to injections into the brain and also into the spleen. The meninges of the brain are capable of supplying the types of mesenchymal cells essential for the growth of lepra bacilli and the spleen is particularly rich in such cells. In patients who show involvement of the nerves, the Hansen bacillus grows in mesenchymal cells within the nerve sheath; it has been mentioned only very rarely indeed as occurring in the ganglion cells.[7] In spontaneous cases of rat leprosy the nerves remain unaffected.

Subcutaneous Injection: A group of animals was injected subcutaneously in order to facilitate the interpretation of the effect of using special routes of inoculation with this particular strain of murine leprosy. Infectious material from a subcutaneous lesion in a rat was suspended in testicular extract and injected subcutaneously into 2 monkeys (*M. rhesus*), 3 rabbits and 4 guinea pigs. Local lesions developed at the site of inoculation but these regressed spontaneously in the course of a few months. Several groups of white mice were injected subcutaneously with essentially similar results; 2 exceptional cases will be described subsequently. Instead of the interpretation that these various species are altogether insusceptible to murine leprosy, it seems probable that they are markedly refractory but not entirely resistant. The small subcutaneous lesions (1 to 2 mm.) in mice ordinarily contain numerous lepra bacilli of normal morphology and their virulence has been demonstrated on several occasions. In two instances a small amount of material from subcutaneous lesions was used successfully for the serial passage of murine leprosy in mice.

Intracerebral Injections: For the first test of intracerebral inoculation a rich suspension of murine lepra bacilli in testicular extract was injected into 26 adult white mice, average weight 20 gm., 2 monkeys (*M. rhesus*), and 8 young rats, average weight 60 gm. The rats received about 1/30 cc. each, the mice about 1/60 cc., and the monkeys ¼ cc. each.

It was not feasible to record complete data concerning all of the

mice. During the succeeding months after inoculation some died of intercurrent disease and the brains and even the viscera were frequently eaten by the survivors. However, observations were obtained at representative periods, including both short and long intervals. One mouse died 24 days after injection and a few intracellular lepra bacilli were seen in smears from the brain. In 1, which died 56 days after injection, cells were seen that were well filled with lepra bacilli; another was killed 79 days after injection and sections of the brain showed well developed lesions of leprosy. At 88 days after injection 1 mouse developed complete paralysis of the hind legs and was killed; smears from the brain showed occasional clumps of lepra bacilli. Especial interest attaches to a mouse that lived for a longer period. One year and 23 days after injection this animal was in an altogether critical condition and was killed. The autopsy showed well marked lesions of leprosy in the brain and extensive metastases widely distributed throughout the body resulting in a massive infection. The conclusion was obvious that this animal was dying of leprosy.

The 2 monkeys remained in good condition for several weeks. By the 37th day after injection 1 of them had developed well marked paresis of the extremities and was found dead 4 days later. Sections of the brain showed definite evidence that leprosy bacilli had multiplied extensively within the mesenchymal cells in the meninges and around the blood vessels. The immediate cause of death might very well have been increased intracranial pressure. The 2nd monkey became progressively weaker and died 2 months after injection. The autopsy findings were similar to those in the 1st monkey but in addition there was a complicating bilateral bronchopneumonia.

Under ordinary conditions rat leprosy usually runs a course of a year or more in its duration. Of the 8 young rats injected intracerebrally, 6 died or were killed when almost *in extremis* at irregular intervals between the 2nd and 4th months after injection, the short course of the disease being attributed to excessive intracranial pressure. Two of the 8 rats lived for 9 months. The extent of metastases depended chiefly on the length of life after injection; in these 2 rats there was a well marked infection of the cord with extensive involvement of the spinal nerves.

For confirmation of the preceding results a second series of

animals was injected intracerebrally with murine leprosy from the subcutaneous lesion of a rat as follows: 22 mice with lepra bacilli suspended in saline solution; and 2 rabbits, 1 monkey and 4 guinea pigs with lepra bacilli suspended in testicular extract.

Eighteen of the mice died within 3½ to 5½ months after injection. The sections showed extensive lesions of leprosy in the brain with metastases commonly in the spleen and occasionally in the liver. None of this group survived for an exceptionally long period, the last mouse dying 6 months and 19 days after injection. One of the 2 rabbits injected with murine leprosy died a week later of an intercurrent infection. The other rabbit died with some slight paresis on the 24th day after injection, and sections of the brain showed histological evidence of the multiplication of lepra bacilli, the exact cause of the paresis being undetermined. One month after injection the monkey died of some unknown cause and the sections also showed early signs of leprosy. The 4 guinea pigs remained well or died of some intercurrent disease and in no case were any lepra bacilli found microscopically. The last of these guinea pigs was killed and autopsied 1¼ years after injection.

Subsequently another group of 6 mice was injected intracerebrally with murine leprosy and each mouse developed a well marked infection. Rabbits, however, have behaved with irregularity. One was sacrificed 4 months after intracerebral injection and smears from the brain showed a few scattered lepra bacilli but there was no evidence of active infection. Another rabbit developed marked paresis of the hind legs 24 days after an intracerebral injection and was chloroformed. A cold abscess had developed in the ventricle on the side of the brain that was injected and smears of the affected tissue showed many lepra cells. Preliminary attempts to obtain serial passages in rabbits have failed.

Injection of Mice in the Spleen: The spleen was exposed by laparotomy under general anesthesia and injected with about 1/16 cc. of a suspension of lepra bacilli from an infected rat. Of 10 mice injected in this manner, 5 died a few hours after the operation. Of the 5 survivors, 1 died 19 days after injection; microscopically sections of the spleen showed several foci of lepra cells. Two mice were killed at later intervals (4 months, 25 days, and 5 months, 22 days) and a massive infection of leprosy was found in the spleen. The 2 remaining mice were allowed to live for

longer periods. In 1 of these animals the abdomen was enormously distended with fluid in the peritoneal cavity, the spleen was enlarged, very hard on palpation and adherent to the peritoneal wall. The skin over this area had broken down, exposing granulation tissue characteristic of leprosy. At this time, 8½ months after injection, this animal was in a critical condition and was killed. At autopsy there was one feature of especial interest. The liver under its capsule and on section was studded with whitish nodular lesions of leprosy of almost pin-head size, reminiscent of the gross appearance, for example, of the liver of an animal dead of bubonic plague. An identical picture was found in the remaining mouse of this group, killed 9 1/6 months after injection.

The intrasplenic injections were repeated with murine leprosy taken from the local lesion in a dilute brown mouse 13 1/5 months after subcutaneous inoculation. A saline suspension of material from this lesion was injected, after laparotomy, into the spleens of 3 white mice. The first death in this group occurred at 1 month and 3 days, and the second death at 4 months and 23 days after injection. Both animals showed a well developed leproma in the spleen with extensive miliary lesions in the liver. In the 3rd mouse a firm tumor was found on palpation of the left side of the abdomen, and this was interpreted as an enlargement of the spleen. The condition of this animal was rather critical and it was killed 4 months and 24 days after injection. There was a definite tumor in the spleen, but the palpable tumor in the abdomen was found to be a leproma (0.75 cm. in diameter) which had developed at the site of the incision in the peritoneum. A similar but smaller tumor (2 to 3 mm. in diameter) was found some distance away in the uninjured wall. It is obvious that after the injection into the spleen there is abundant opportunity for leakage of inoculum from the site of the injection, thus soiling the peritoneum with lepra bacilli.

Inoculation into the spleen often leads to the development of localized nodules at the site of injection accompanied by a widespread infection of the liver which in some cases has been more extensive than in the spleen itself. Metastatic infection of the spleen results in uniformly distributed miliary lesions which show but little tendency to extend to the liver. When the spleen is traumatized by the sudden injection of a rather large inoculum,

lepra bacilli might easily find their way freely and promptly through the veins into the liver. The injection into the spleen would become in effect a simultaneous injection of the spleen and liver. Two mice were injected directly in the liver with a strain of murine leprosy which had been maintained in mice for 16 months. In both cases extensive hepatic lesions developed. Also, in 1 of the 2 mice the omentum was studded with lepromas, this animal having been killed while still in good condition at an interval of 6 months after its injection.

Intraperitoneal Injection: In exceptional instances it was noted that white rats escaped infection after the intraperitoneal injection of murine lepra bacilli and on one occasion we lost this strain of leprosy through the failure of a rat to develop any lesions after inoculation by this route. Injections by the intraperitoneal route were omitted in our earlier observations on the susceptibility of mice. However, upon injecting mice in the liver or in the spleen it has already been noted that there is usually some gross leakage of the inoculum into the peritoneal cavity and in some of these animals lepromas have developed in the parietal peritoneum and in the omentum. Accordingly, a group of 17 white mice of mixed breed was injected intraperitoneally with murine lepra bacilli taken from an infected rat. These animals were sacrificed 6 to 7 months after injection. Fifteen showed gross changes, notably miliary lesions of the spleen and of the liver. Frequently the omentum was studded with lepromas. In several instances the epididymis was the seat of an exceptionally massive infection. The lungs usually escaped any extensive involvement. Two mice showed no gross lesions, but a careful examination of smears from the viscera and from the peritoneal wall of each resulted in the finding of a few isolated acid-fast bacilli. Similar infections were reported recently in "German mice" by Watanabe [8] after intraperitoneal injection with murine leprosy.

Miscellaneous Routes of Injection: The counterpart of the more or less pure forms of nerve involvement by the Hansen bacillus in man does not occur in animals infected with murine leprosy and this type of lesion failed to develop under the following experimental conditions. Two monkeys (*M. rhesus*), and 2 rabbits were injected under general anesthesia in the sciatic nerve with a rich suspension of murine lepra bacilli. The final examinations were

made 8 to 24 months later. In each instance the nerve was normal in its gross appearance and preparations from the site of injection showed no acid-fast bacilli. A 3rd monkey was injected in the great cistern with murine leprosy 8 months ago and at present this animal appears to be in good health.

In generalized infections with murine leprosy the kidney, except for the invasion of its capsule, ordinarily though not invariably escapes infection. Three rabbits were injected in the kidney by direct puncture through the abdominal wall. These animals remained well and were sacrificed at intervals of 3 to 6 months. In 2 of these a few isolated clumps of acid-fast bacilli were found in sections of the kidney but there was no indication that an infection had been established.

Serial Passage: Murine leprosy has been maintained continuously since 1934 by passage in white mice of mixed breed, eight transfers having been made at irregular intervals by various routes of inoculation during a period of 4 years. Subcutaneous injections were used for the second and third passages and produced only small local lesions which did not suggest that this strain during the course of more than a year had become adapted to the mouse in any striking manner. Inasmuch as the subcutaneous lesions afforded only a minimal amount of infectious material the subsequent passages were carried out by other routes of inoculation. The fourth passage was made by direct puncture through the abdominal wall into the spleen without incising the skin. The spleen was injected successfully by this technique in several instances, but in some cases this procedure resulted merely in an intraperitoneal injection. The details of these passages are given in the accompanying table.

Mice of Known Genetic Stock: The use of animals of a high degree of genetic uniformity reduces the number of variables that one encounters in using mixed breeds. Dr. C. C. Little, Director of the Jackson Memorial Laboratory of Bar Harbor, Maine, has supplied us with several strains of inbred mice designated as, "M" leaden, "C 57" black, "D" dilute brown and an inbred albino strain. Dr. L. T. Webster of the Rockefeller Institute kindly sent us a stock of the Swiss strain of white mice which is now in common use in the study of a variety of infections. Preliminary observations, extending over more than 3 years, indicate that some of

these strains, particularly the ones designated as "dilute brown" and "C 57" black, might be used with fair success for the routine propagation of murine leprosy by subcutaneous injection. Frequently, tumors that are readily visible and palpable (0.5 to 1 cm. in diameter) have developed at the site of inoculation under the skin, and extensive metastases, particularly to the liver and spleen, have led to a progressive disease. Infections running a similar course have been noted with considerable regularity in mice of the

TABLE I

Serial Passage of Rat Leprosy in White Mice of Mixed Breed

Number of passage	Tissue of infected mouse used for injection	Route of inoculation	Interval between passages
First	* ——	Intracerebral	——
Second	Brain and lung	Subcutaneous	1 year, 23 days
Third	Subcutaneous lesion	Subcutaneous	8 months
Fourth	Subcutaneous lesion	Intrasplenic	2 months, 14 days
Fifth	Spleen	Intracerebral	2 months, 21 days
Sixth	Brain	Intracerebral	5 months
Seventh	Brain	Intracerebral	7 months
Eighth	Brain	Intraperitoneal	6 months

* Subcutaneous lesion of white rat.

Swiss strain. The extensive, infiltrating subcutaneous lesions commonly occurring in rats have not been seen at any time in mice. Murine leprosy, which had been carried through two passages by subcutaneous injection in dilute brown mice during the course of 1 year and 2 months, was then injected into a white rat and characteristic lesions developed. It was not until the 3rd year of our experience with white mice of mixed breeds that any extensive infections were observed after subcutaneous injection of murine leprosy. In 2 mice from a group of 18, the lesion under the skin was insignificant in size (1 to 2 mm.) but the metastases in the liver and spleen and the course of the disease were altogether similar to the extensive infections observed in some instances in mice of inbred strains. The infrequency of this result is in contrast with the uniformity with which severe infections developed after other routes of inoculation. Lepromatous material taken directly from an infected rat was used for the inoculation of this group of mice.

Controls with Cultures of Acid-Fast Organisms

The conservative interpretation in the literature on the transmission of human leprosy to lower animals is based on the persistence of intact lepra bacilli at the site of injection over long periods. Even in the absence of any multiplication, their presence in the tissues incites a reaction that may imitate a true infection. Several groups of mice were injected with strains of non-pathogenic acid-fast bacilli, using *B. smegmatis*, a culture from timothy hay, one from butter, and a stock culture of a chromogenic organism labelled merely *B. lepra*. These microorganisms presumably have little or no ability to multiply in tissues. Heavy suspensions were made in testicular extract and injected intracerebrally, using 8 mice for each culture. In the first few days after injection many of the animals showed signs of excessive intracranial pressure and about one-third of them died; some of the survivors were sacrificed at later intervals for histological study.

The efficacy of the intracerebral route of inoculation was tested with cultures of the tubercle bacillus, using the avirulent BCG and a moderately virulent H 37 strain; 5 mg. of the latter, weighed in the moist state, infected guinea pigs with regularity. A heavy suspension of the avirulent culture of the human tubercle bacillus was made, using 40 mg. of the moist growth to 2 cc. of an extract of normal guinea pig testis. This was injected intracerebrally into 4 guinea pigs and into 9 white mice; none of these animals developed any evidence of infection. A moderately turbid suspension of the H 37 strain with the addition of testicular extract was injected into 9 white mice intracerebrally and into 9 controls subcutaneously. The mice injected intracerebrally died or were killed at intervals of 6 days to 5 months and 18 days. Lesions occurred in the brain which presented many of the features of murine leprosy. Moreover, in 2 of these mice macroscopic tubercles developed in the lungs and in 2 of the remaining mice the sections showed the presence of pulmonary tuberculosis. In 1 mouse, groups of macrophages laden with acid-fast bacilli were found in the spleen. The control mice injected subcutaneously died or were killed within 1 to 6 months after injection. No lesions and no acid-fast bacilli were found either at the site of injection or in the viscera.

Human Leprosy

During the course of this work we have had an opportunity to study one patient with leprosy, the lesions being of the nodular type in a rather early stage. A cervical lymph gland was excised under local anesthesia and ground lightly with sand in physiological saline, with the addition of testicular extract. The resulting suspension was not especially rich in lepra bacilli. Fifteen mice were injected intracerebrally. Two monkeys (*M. rhesus*) were injected intracerebrally and intradermally in the eyebrow and also subcutaneously. The mice were sacrificed at intervals varying from 2 weeks to 1 1/6 years. Four were autopsied 2 to 3 months after injection and smears of the meninges showed scattered lepra bacilli, but the inoculation of such material into the brains of white mice produced no infection.

The results in the monkeys were more interesting. The local reactions developing at the site of injection into and under the skin were insignificant and the animals remained in excellent health. In 1 of the monkeys a unilateral enlargement of the femoral glands was noted 1 year and 5 months after injection and some of these glands were removed under local anesthesia. Smears of a minute lesion showed large numbers of lepra bacilli but a further search of these glands failed to provide any material at all suitable for the inoculation of animals. This monkey died 1 year and 6 1/3 months after injection and the autopsy revealed no cause of death. No lepra bacilli were found in smears of the meninges, the viscera or of the superficial glands.

The 2nd monkey behaved in a manner analogous to that of its companion. One year and 4 months after injection this monkey was in a critical condition and was chloroformed. No gross lesions were noted at autopsy. Lepra bacilli were found in the glands of one axilla in moderate numbers and also in smears of the pia mater. There was nothing at all comparable to the massive infection which was seen in monkeys and mice injected with murine leprosy. The axillary gland was ground with sand and suspended in saline. Injections were made intracerebrally into monkeys (Nos. 3 and 4) and into mice, and also subcutaneously into 2 guinea pigs for the exclusion of tuberculosis. The guinea pigs were subsequently

killed and no lesions were found at autopsy. No infections with leprosy have occurred in the mice.

The observations on the monkeys (Nos. 3 and 4) consisted chiefly of the examination of superficial lymph glands removed by biopsy under general anesthesia. In Monkey No. 3, 7 months after injection an increase in size of the right inguinal glands was noted. Some of these glands that showed a few good clumps of acid-fast bacilli were inoculated into 2 guinea pigs and both animals remained free of any sign of tuberculosis. This monkey was in a semimoribund condition 11¾ months after its inoculation and was chloroformed. No gross lesions were found at autopsy. Smears of the dura mater showed a few acid-fast bacilli but none were seen in smears and sections of the lymph glands.

Some small glands in the right axilla were removed from the remaining monkey (No. 4) 11 months after its inoculation. Smear preparations showed occasional acid-fast organisms entirely consistent in their morphology with the Hansen bacillus. After an additional interval of 2¾ months a mass of about 12 small glands of from 2 to 8 mm. in length was removed from the left inguinal region. One of these glands showed a few acid-fast organisms and small portions of it were implanted in the brain of 2 white rats. On the following day this monkey was chloroformed. Occasional acid-fast bacilli were seen in smears of the pia mater and portions of the pia were implanted in the brain of 6 white rats, but the acid-fast microorganisms were very sparse and it is by no means certain that each animal actually received lepra bacilli. These inoculated rats, 8 in all, remained in good health and have been sacrificed at intervals of 10 to 15 months. In 2 of these animals smears from portions of the meninges showed rare acid-fast bacilli and in a 3rd smears from the choroid plexus contained many clumps of acid-fast microorganisms which were evidently the Hansen bacillus.

According to these results monkeys have shown more susceptibility to murine than to human leprosy. This situation is not necessarily illogical for the same conclusion might conceivably apply to man. Volunteers have frequently remained well after inoculation with human lepromatous lesions. These failures in the experimental transmission of human leprosy to its natural host emphasize the difficulty of establishing a progressive disease in

foreign species. The ease with which murine leprosy can be successfully transmitted by inoculation stands in distinct contrast with the behavior of human leprosy. However, intensive study has in general revealed additional similarities rather than emphasizing the differences between these two diseases.

Summary and Conclusions

1. Inoculation of murine leprosy into the brain resulted in the development of progressive lesions in monkeys (*M. rhesus*), in rabbits, in white rats and in ordinary white mice, but not in guinea pigs.

2. White mice of mixed breeds were also readily infected by injection in the spleen, the liver or the peritoneal cavity, but subcutaneous injection usually resulted only in an abortive infection. Mice of some inbred strains frequently developed well marked local lesions on subcutaneous injection and subsequently extensive metastases occurred.

3. Murine leprosy has been maintained by serial passage in white mice of mixed breeds for 4 years. Various routes of inoculation have been used. Massive infections have developed, providing excellent material for experimental purposes.

4. The bacillus of rat leprosy produced infection in the murine rodents, white rats and white mice, according to the manner of a microorganism developing in a favorable host, but the circumstances for its multiplication were apparently less favorable in rabbits and monkeys.

5. Monkeys (*M. rhesus*) were kept under observation for more than 1 year after inoculation with human lepromatous material. Lepra bacilli were seen in small numbers in smears of inguinal glands. Inoculations were made to other monkeys and a similar low grade infection developed during a period of more than a year. Portions of the pia mater of one of these monkeys were implanted intracerebrally in white rats; 15 months later many acid-fast bacilli were found in the brain of 1 of these rats. Briefly, human lepra bacilli were maintained in animals for a period of 3¾ years but no progressive disease and no active lesions developed.

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HISTOLOGICAL AND CYTOLOGICAL STUDIES OF MURINE LEPROSY *

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Histological studies of localized lesions developing at the site of inoculation in the various species of animals employed have been invaluable in determining whether or not true infection, in the sense of a progressive multiplication of the organisms introduced, had taken place. Animals showing generalized infection furnished excellent material for the study of the origin and development of the cells (lepra cells) which act as hosts for the lepra bacilli in various organs and tissues. The study of early lesions is particularly important for this purpose.

In rats the infection tends, for a time, to remain localized at the site of inoculation, but in all instances distant organs were eventually involved. This metastatic involvement occurs in an irregular manner, but sooner or later, if the animal lives long enough, nearly every organ and tissue may be massively involved. As has been brought out above, massive infection by direct injection into an organ usually resulted in the rapid development of extensive lesions there. The kidney was comparatively resistant to infection perhaps because this organ contains fewer mesenchymal cells of the type that readily become infected. No histological or cytological differences were observed between the local and metastatic lesions produced in mice and those produced in rats. Certain peculiarities of the lesions developing in monkeys and in rabbits will be brought out later.

In general, the pathological changes are closely parallel to those in human leprosy. Lepra cells, the cytoplasm of which is densely packed and often distended with bacilli, increase at the expense of normal tissues which undergo extensive pressure atrophy. Cell nuclei, which are never invaded, are usually normal in appearance,

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but occasionally are compressed and pyknotic. They are often flattened along the periphery of the cell, giving a "signet ring" appearance like that of the normal fat cell. The vacuolated or foamy appearance of the cytoplasm of infected cells, which is characteristic of the human lesion, has only very rarely been observed in our material. Giant cells are often present in large numbers.

The observations made are in harmony with the belief that murine lepra bacilli are capable of multiplying only within cells. Extensive areas of cell degeneration within lepra nodules, resulting probably from infarction, are frequently seen. In such areas extracellular bacilli set free by the dissolution of their host cells persist in enormous numbers apparently because of their great resistance to autolysis and heterolysis. Such bacilli are often disseminated in tissues during the process of preparing the sections. In no instance has the distribution of organisms appeared to justify the assumption of extracellular multiplication.

Lepra bacilli are commonly believed to be capable of growing only in mesenchymal cells of a certain type. Various workers, including Oliver,[1] Henderson,[2] and Lowe,[3] have concluded that the lepra cells are derived from the cells that constitute the reticuloendothelial system. This concept, with certain exceptions that will be noted below, is in general supported by the present studies. In taking up lepra bacilli the cells of the reticuloendothelial system are merely carrying out one of their normal functions, the phagocytosis of lipoid structures that are physiologically useless or detrimental to the organism. It is the ability of the ingested bacilli to multiply within these cells, instead of being metabolized there, that makes possible the development of progressive and eventually fatal lesions. As the lesion progresses new lepra cells are formed, not only from the local mesenchymal cells, but also by mitotic division of preëxisting lepra cells, so that the process is in some respects analogous to a neoplasm. Lepra cells are found in mitosis with sufficient frequency to suggest that the organisms, or some product of their metabolism, stimulate cell division. The lepra cells do not, however, acquire any of the morphological characteristics of malignant tumor cells.

Mesenchymal Tissues: Cells that are commonly considered to be members of the reticuloendothelial system, such as the Kupffer

cells of the liver and the cells lining sinuses and forming the reticulum of spleen, lymph nodes and bone marrow, were found, by a study of the earliest lesions, to be initially infected. Isolated Kupffer cells, for example, were often infected (Fig. 3) and frequently distended with bacilli. Ordinary vascular endothelium, including that of arterioles, venules and precapillaries, was never found infected and was almost invariably in a flattened quiescent state, even when these vessels were within nodules composed almost entirely of lepra cells. Smooth, striated and cardiac muscle fibers likewise appeared invariably to escape infection, although often studied in locations where they must have been adequately exposed. The same statement may be made regarding cartilage cells and also serosal and meningeal lining cells. The observations regarding the latter two types of cell are particularly valid, because these cells were very frequently seen as single rows of flattened, uninfected cells overlying tissue composed almost entirely of lepra cells. Polymorphonuclear leukocytes, which were present in the lesions in relatively small numbers, often contained bacilli but never in such numbers as to suggest multiplication within these cells. Cells that could be recognized as lymphocytes, plasma cells and eosinophiles were always free from infection. It will be noted that the cells thus far described which were found infected are precisely those commonly considered as members of the reticuloendothelial system on the basis of their reaction to intravenously injected finely divided foreign substances.

The question of the reaction of fibroblasts and fat cells to infection could not be answered definitely by these studies. Large areas of ordinary connective and adipose tissue often became replaced by tissue which appears to be composed entirely of lepra cells, with a network of thin walled blood vessels. It seems probable that both the fibroblast and the lipoblast may eventually become infected, but one cannot rule out the possibility that they may disappear by pressure atrophy at the expense of lepra cells originating from histiocytes and by mitotic division of preëxisting lepra cells. The mesenchymal cells immediately adjacent to the sarcolemma of striated muscle fibers, regarded by some workers as specialized cells responsible for the formation of sarcolemma, become infected very early (Fig. 6).

In early lesions involving the testis isolated groups of interstitial

cells were often found infected (Fig. 2). This observation is in harmony with the view that certain of these interstitial cells belong to the reticuloendothelial system. In late lesions the interstitial cells appeared to become uniformly infected; here again, perhaps, as a result of pressure atrophy of those cells that were not originally invaded. After intracerebral inoculation infection commonly spread in the subarachnoid tissue over the entire surface of the brain, reaching the interior of the brain by following along the walls of the blood vessels (Fig. 1). In several instances infection also spread in the subarachnoid tissue of the spinal cord and produced extensive lesions of the cauda equina and peripheral nerves. Atrophy of the nerves was evidently due to the conversion of the mesenchymal cells into lepra cells and not to actual invasion of the nerve fibers. Ganglion cells were not found infected.

In the kidney, where infection was infrequent and rarely progressed to a point where lesions were grossly visible, the cells initially infected were mesenchymal cells in glomeruli and in the walls of intertubular capillaries and larger vessels. In the adrenal isolated mesenchymal cells or small clusters of mesenchymal cells were frequently infected. Such cells were found in both cortex and medulla but were, in several instances, most numerous at the junction of cortex and medulla. In bone marrow both extravascular and intravascular mesenchymal cells appear to be initially infected. Eventually by the proliferation of such cells the marrow becomes entirely replaced by lepra cells, the marrow spaces become greatly enlarged, and there is marked atrophy and disappearance of bony trabeculi (Fig. 7).

Epithelium: In general, it may be said that epithelial cells of all types were relatively resistant to infection. Liver cells, pancreatic acinar and islet cells, gastro-intestinal epithelium, and mucous gland and bronchial epithelium were invariably found to be free from infection, even when embedded in nodular masses of heavily infected mesenchymal cells.

In rare instances ependymal cells and neuroglia cells appeared to be lightly but definitely infected. In 1 rat lepra bacilli were found within epithelial cells of the epidermis in such large numbers as to indicate that intracellular multiplication had taken place. This rat was inoculated intracerebrally and extensive metastases

developed in the cervical lymph nodes. Cells forming all three layers of the epidermis overlying these nodes were infected, and localized multiplication of these infected cells had obviously taken place, sometimes producing small raised nodules with hyperkeratinization and sometimes causing localized areas of downward growth into the corium. In 1 mouse, also, the epithelial cells lining the tubules of the testes (Fig. 2) and the ducts of the epididymis were extensively invaded, so that the infected structures could easily be selected with the low power lens. This mouse was injected in the spleen with a suspension of lepra bacilli and showed extensive secondary involvement of the inguinal nodes and testis.

These observations were so infrequent that they should be considered as exceptions rather than the rule. Their chief importance lies in their suggestion that the relative resistance of epithelial cells to infection depends on their feeble phagocytic powers or on the inability of the bacilli to enter them, rather than upon intracellular conditions that are inhibitory to the growth of lepra bacilli.

Marchoux and Sorel [4] in 1912 studied the skin lesions of murine leprosy and noted in certain areas the presence of lepra bacilli in epithelial cells, an observation that has not attracted the attention of subsequent workers.

Lesions Produced in Monkeys and Rabbits

Although definite evidence of progressive local and metastatic infection of rat leprosy in both monkeys and rabbits was obtained there is reason to believe that *B. leprae murium* grows less readily within the cells of these species than in those of rats and mice. In general, infected cells contained fewer organisms and infected tissues, stained by the Ziehl-Neelsen method, were never definitely red in color when examined with the unaided eye, as they commonly were in rats and in mice. Although numerous miliary lepromas were found in the livers of monkeys after intracerebral inoculation, lepra bacilli were scarce in such lesions and were found only after prolonged research. The cells composing these lepromas also differed from the lepra cells seen in rats and mice in that they showed the cytoplasmic vacuolization which is characteristic of the human lepra cell.

Lesions Produced by Intracerebral Injection of Other Acid-Fast Organisms

Mice surviving the intracerebral injection of non-pathogenic acid-fast organisms never showed metastatic lesions. The organisms introduced called out many macrophages and neutrophiles. Organisms were found in large extracellular masses, but they were also taken up in large numbers by both macrophages and neutrophiles. A few days after injection these organisms showed evidence of degeneration, and after about 3 weeks they had entirely disappeared, although the inflammatory reaction was undiminished in intensity. No evidence of multiplication of these non-pathogenic organisms in the tissues was obtained, and the lesions produced by them could be readily distinguished from those of leprosy.

Avirulent tubercle bacilli (BCG) persisted longer in the brain, and several weeks after injection were found in large numbers in macrophages, presenting a picture more closely resembling that of leprosy. Organisms never distended the cells greatly, however. It was not possible to determine whether or not multiplication of these organisms had occurred in the tissues, but metastatic lesions did not develop.

With the virulent strain of the tubercle bacillus (H 37) lesions were produced in mice which simulated even more closely those of leprosy and which were accompanied by extensive metastatic lesions in the lungs (Fig. 8) and spleen. In these metastatic lesions organisms were present in enormous numbers and were largely within macrophages. These tuberculous lesions were not caseous and were often practically identical with those of leprosy, although the tubercle bacilli themselves could be identified because of their larger size and morphological characteristics. These metastatic tuberculous lesions developed only after intracerebral inoculation; subcutaneous inoculation of similar doses did not result in localized or metastatic infection.

Summary

Lepra cells in murine leprosy are derived largely from mesenchymal cells belonging to the reticuloendothelial system. Exceptionally, however, epithelial cells, specifically those of the epidermis, testicular tubules and epididymis, became distended with

lepra bacilli. This observation suggests that the relative resistance of epithelial cells to infection may depend on the inability of lepra bacilli to enter them, rather than on intracellular conditions unfavorable to their growth.

In infected rats and mice surviving for a long time the tissues of practically all organs were extensively replaced by non-vacuolated lepra cells distended with bacilli, but kidney tissue contained relatively few of these cells.

In the progressive local and metastatic lesions produced with *B. leprae murium* in rabbits and monkeys lepra cells were often vacuolated and acid-fast bacilli were much less numerous in these lesions than in those produced in rats and mice, and were found only after prolonged search.

Non-pathogenic acid-fast bacilli, injected intracerebrally, were taken up by macrophages and neutrophiles, but disappeared from the lesions in a few weeks, never producing metastatic lesions.

Virulent tubercle bacilli, although innocuous when injected subcutaneously into mice, produced progressive and metastatic infection when injected intracerebrally into these animals. The lesions were non-caseating and the tubercle bacilli were found largely within macrophages, so that these lesions closely resembled those of leprosy.

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DESCRIPTION OF PLATE

All sections were prepared from tissues fixed in 10 per cent formalin in 95 per cent alcohol and stained by the Ziehl-Neelsen method.

PLATE 122

FIG. 1. Cerebral cortex of mouse after intracerebral injection of murine lepra bacilli showing phagocytic cells laden with acid-fast bacilli filling the perivascular space. The vascular endothelium is not infected. × 570.

FIG. 2. Mouse testis after intrasplenic injection of murine lepra bacilli. Note infection of tubular epithelial cells and patchy distribution of lepra cells in the interstitial tissue. × 400.

FIG. 3. Liver of mouse after intracerebral injection of murine lepra bacilli showing infection of isolated Kupffer cells. × 1300.

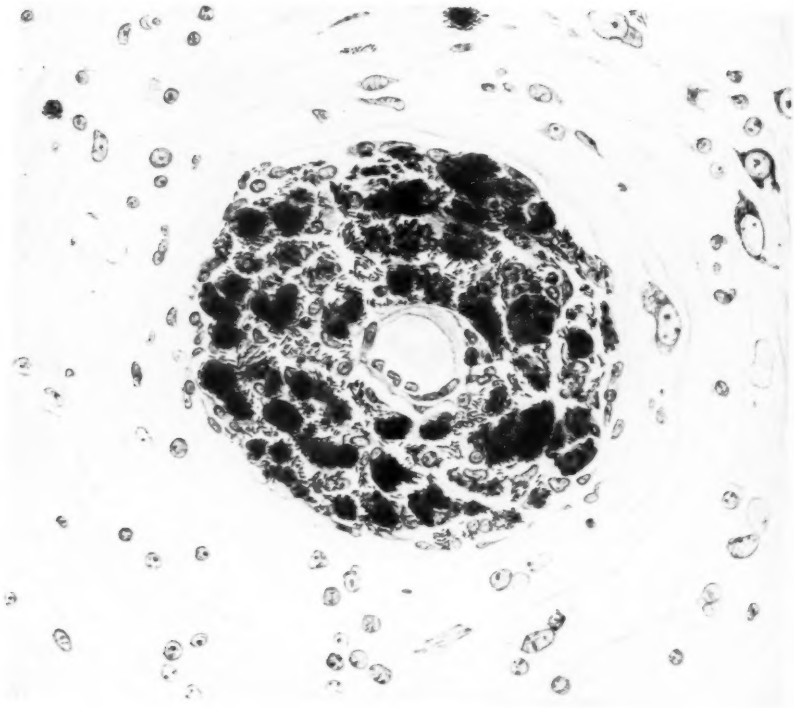
FIG. 4. Spinal nerve of rat after intracerebral injection of murine lepra bacilli. Lepra cells within the nerve bundle are derived from the mesenchymal cells there. The nerve fibers are not infected but disappear from pressure atrophy. × 570.

FIG. 5. Stomach of mouse after intracerebral injection of murine lepra bacilli showing lepra cells of mesenchymal origin. × 570.

FIG. 6. Striated muscle in rat leprosy showing infection of mesenchymal cells adjacent to the sarcolemma. × 570.

FIG. 7. Skull of rat after intracerebral injection of murine lepra bacilli showing accumulation of lepra cells in marrow space and consequent atrophy of bone trabeculae. × 570.

FIG. 8. Lung of mouse after intracerebral injection of virulent tubercle bacilli (H 37). The alveoli and alveolar walls contain many macrophages laden with tubercle bacilli, simulating the appearance of lepra cells. × 570.



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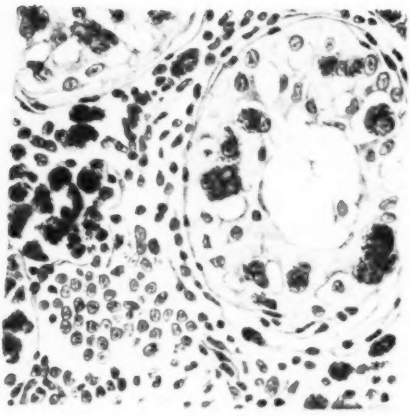

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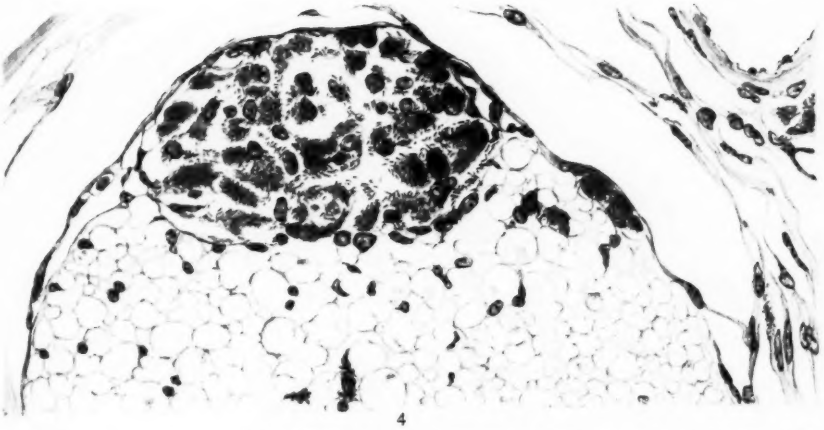

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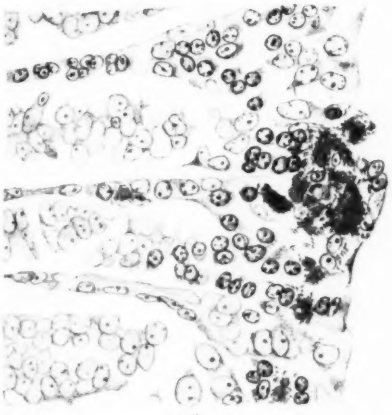

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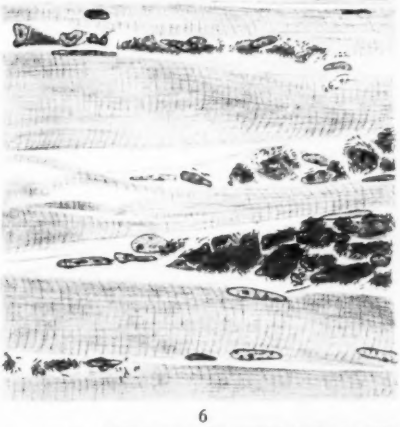

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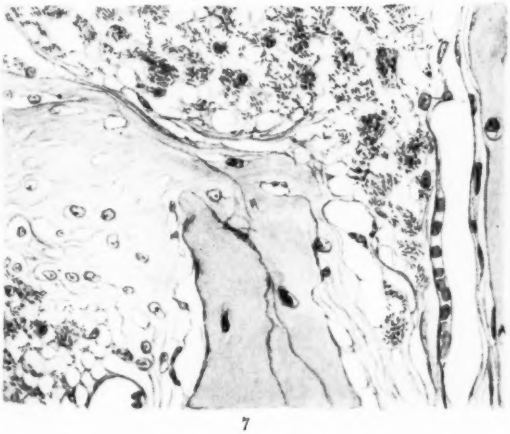

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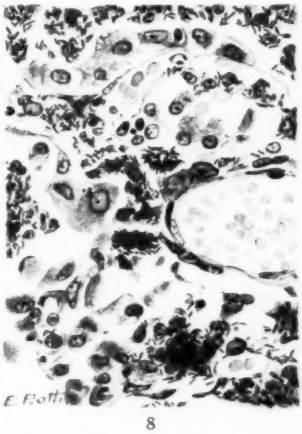

8

Studies of Murine Leprosy

UNIV. OF MICH.

MORPHOLOGICAL VARIATIONS OF TUMOR CELLS *

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Routine histological examination of a large group of malignant epithelial tumors during the past five years revealed in a number of instances distinct variations of individual cells or groups of cells in a single tumor. These variations were so extensive as to render the classification of these tumors difficult. In a series of cases it was not possible to classify these tumors, and diagnoses of "predominantly one type of tumor," such as "predominantly squamous cell carcinoma" had to be made. It was thought of interest to study in detail the histological appearance of such tumors, to see whether or not their complexity could be explained on the basis of collision or combination tumors and to see how such variations interfere with their gradations and affect the prognosis for the patient.

MATERIAL AND METHODS

This study is based on a selected group of various tumors derived from the routine material sent to the histological laboratory from the tumor clinic and the various departments of the Michael Reese Hospital. Those tumors were selected for study which in routine examination showed more than one apparent type of tumor cells. Fifty such tumors were studied. The majority were malignant tumors arising in the skin or mucous membranes, such as the oral cavity, larynx, esophagus, rectum and uterus. Blocks from these tumors were cut, and frozen and paraffin sections were made. In some instances serial sections were cut from individual blocks. Iron hematoxylin without counterstains and the hematoxylin-eosin stain were used, in addition to various special stains whenever deemed necessary.

RESULTS

Carcinoma of the Cervix and Uterus: There were 20 cases of carcinoma of the cervix and uterus where a definite diagnosis of

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carcinoma consisting of one type of cells could not be made. Most commonly this occurred in the type of carcinoma consisting mainly of transitional or spindle shaped cells in addition to other epithelial structures. These tumors arose either from the portio uteri or from the cervix and were apparently rather young, slightly ulcerated, but not well defined. Histologically there were fields of typical mature squamous cells with keratinization in addition to nests of squamous cells which were unevenly stained, showed atypical mitoses and were undoubtedly malignant. Other fields revealed accumulations of more cuboidal or spindle shaped cells resembling those seen in the basal cell layer of the epidermis; either between these cells or close by, thin spindle shaped nuclei with hardly any cytoplasm were recognizable, these cells resembling connective tissue cells. Transitional forms from the basal cell type of cells to the latter were often encountered. Other fields of the same tumor showed small, round or oval shaped cells with deeply stained nuclei. All these types of cells exhibited various degrees of anaplasia and atypical mitotic figures. The outstanding feature was the fact that the various types of cells were intermingled with each other so that squamous cell islands with or without keratinization were often surrounded by thin spindle shaped cells, or small anaplastic cells, and often transitional cells of the basal cell variety were found adjacent to the squamous cells. In individual sections it was sometimes possible to designate which of the tumor cells was predominant, but in the sections cut serially a diagnosis was barely possible.

In 3 instances sections of the tumors revealed large adenomatous structures which showed unquestionable evidence of malignancy. In addition to these structures there were more or less circumscribed areas consisting of squamous cells and transitional cells which in general varied in size, shape and staining quality and also contained atypical mitotic figures. These islets of squamous and transitional cells were present in many foci, which, as serial sections revealed, did not communicate with one another. They also were not particularly pronounced at the edges of the adenocarcinomatous structures.

Carcinoma of the Skin: There were 11 cases in this series where a diagnosis of carcinoma consisting of one type of cell could not be made. Clinically these were as a rule early tumors, as far as the

history was concerned. Most commonly the face was involved and the lesions on gross appearance and location were rather typical of the basal cell type of carcinoma. Histologically also there were fields characteristic of basal cell carcinoma. Nests of epithelial cells, polyhedral, slightly cuboidal and spindle shaped, and often arranged in elongated groups, invaded the subcutaneous tissue. These were distinctly separated from the overlying epidermis. The nuclei were large, vesicular with relatively large nucleoli, and the cytoplasm was scant. Here and there small empty cystic spaces were encountered lined by groups of tumor cells. The overlying epidermis, when not ulcerated, was often hypertrophic and hyperkeratinization was frequent. However, in the midst of the basal cells just described a number of horny pearls were noted and in other fields typical squamous cells or prickle cells with intercellular bridges were encountered adjacent to the basal cell nests. The squamous cells varied only slightly in size and mitotic figures were occasionally noted. Often these squamous cells were found in the midportions of the carcinoma, while the basal cell structures formed the peripheral portions of the tumor. Occasionally cystic structures, as described above, which were similar to those seen in the adenoides cystic variety of carcinoma, typical basal cells and squamous cells with keratinization were found in a single section. By serial sections it could be shown that the squamous cells were nowhere continuous with the squamous cell layer of the epidermis.

Melanotic Tumors: Although it is well known that melanotic tumors vary in different stages and that the structures of malignant and melanotic neoplasms and their metastases present wide variations, two tumors which to a large extent consisted of melanotic cells may be cited. One, obviously a malignant melanotic tumor found in the skin of the leg, measuring grossly 3 by 6 cm. in diameter, histologically showed fields consisting of large spindle shaped cells with large nuclei and dark brown intracellular and extracellular pigment granules. The nuclei were dark and oval. These cells resemble those seen in spindle cell sarcomas. In other fields the cells were large, square or polygonal, with large, lightly stained vesicular nuclei. These two cell types did not at all resemble each other. In the second tumor, which was benign, the tumor cells were rather small and the cytoplasm not well defined, the cells forming a sort of syncytium. Intracellular and extra-

cellular pigment granules were abundant. In addition, areas of squamous cells with or without keratinization were found in the midst of the tumor. Other fields revealed the typical basal cell types of tumor cells. Again, the various cell types were found in the midportion of the tumor and not confined to the peripheral regions. In short, the tumor consisted of nevus cells, squamous cells and basal cells.

Carcinoma of the Lip, Mouth, Epipharynx and Esophagus: There were 11 cases of carcinoma of the mouth and esophagus. Three of the latter were autopsy specimens. Histologically squamous cells were seen with varying amounts of keratinization, the latter much more rare in the esophageal carcinomas. The additional cell type here again was the basal cell variety, best described as spindle or oval cells similar to those seen in the mucosa of the urinary bladder (transitional cells). These cells were arranged in cords and rows invading the submucosa throughout. Many fields of the tumors of the esophagus consisted entirely of these types of cells. Others again showed these cells intermingled with malignant squamous cells. All the cells showed evidence of anaplasia and mitotic figures were abundant.

As far as the tumors of the epipharynx are concerned reference is made here to the tumors investigated by Salinger and Pearlman [1] which also came under my observation. In a number of instances three pathologists, of whom I was one, disagreed as to the type of tumor, whether it was a squamous cell carcinoma, transitional cell carcinoma or lymphoepithelioma. This apparently was due to the fact that various structures, each characteristic of one of these tumors, were present in the individual tumors. According to which of the various constituents was thought to be predominant, the various respective diagnoses were made.

Rectal Tumors: There were 6 cases in this series in which the histological examination also revealed apparently quite different structures. One was typically adenomatous, with many small or larger tubules, lined by one or two layers of cuboidal cells exhibiting atypical mitotic features and distinctly infiltrating the deeper layers. In addition there were epithelial cells arranged in clusters. These cells were polygonal or more or less round, also showing definite evidence of malignancy. These cells did not form a separate entity but were seen scattered throughout the adenomatous

structures. Occasionally areas of keratinization surrounded by tumor cells were seen.

Discussion

Summarizing the findings it may be repeated that in a number of carefully studied tumors arising predominantly in the skin or mucous membranes, not a single type of tumor cells but two or more apparently different types of tumor cells were found. The incidence of various types of tumor cells in malignant conditions of the cervix uteri was noted by a number of investigators. As a matter of fact the grading of cervical carcinoma is based on the recognition of these various cell types. Chambers [2] speaks of "various types of squamous cell carcinoma" and in classifying them as "adult, spindle, keratinized, transitional," and so on, types, he describes all the types of tumors encountered. The principal question involved, however, is whether or not the anaplastic type and transitional type of carcinoma are squamous cell carcinoma or are primarily transitional carcinoma. There are many cases on record of simple squamous cell carcinoma, of anaplastic carcinoma without squamous cells, and of transitional cell carcinoma without squamous cell features. Also, in comparing cervical carcinomas with skin carcinomas, it seems evident that just as the basal cell carcinoma and the squamous cell carcinoma constitute definite entities, so the transitional type of tumor of the cervix, as of any other mucous membrane, is a special, clearly defined entity, apparently the corollary of the basal cell tumor arising in the epidermis. In other words it seems that the squamous cell carcinoma of the cervix is one type of tumor, and the transitional cell carcinoma is another. As the result of careful examination of malignant tumors because of the interest in the grading of tumors (Chambers [2]), and also as a result of this study, it must be emphasized that there are in addition to these two clear-cut tumors a number of tumors arising in the cervix which show the characteristics of both squamous and transitional cells.

An interesting group of carcinomas of the uterus are the so-called adenoacanthomas, 3 of which are included in this series. These tumors, apparently rare (Stein and Torek [3]), may arise in the portio uteri, cervical canal or in the endometrium. Because of the presence of malignant adenomatous structures and squamous

cells the term adenoacanthoma is used. It is apparently significant that the squamous cells are found more or less in the form of islands in the midst of the tumor. This would speak against the belief of metaplasia of cylindrical tumor cells into squamous cells, because such a metaplasia would rather involve one area instead of appearing in minute regions here and there throughout the adenomatous structures. It seems more likely that these tumors arise from regions which *a priori* reveal both glandular structures and islets of squamous cells, as described by Meyer.[4]

The skin tumors in this series consist mainly of squamous cells and basal cells. It seems that islands of squamous cells and keratinization are more frequently present in the basal cell carcinoma than *vice versa*. Ewing [5] classifies epidermoid carcinoma as adult hornifying squamous carcinoma, transitional cell carcinoma and basal cell carcinoma, indicating three distinctly different types. However, he states that in rare cases of basal cell carcinoma the cells may assume pavement characteristics and spines; hornification and pearl formation may appear in traces. Geschickter and Koehler [6] state that Grade IV squamous cell carcinoma resembles a basal cell carcinoma on close examination. They also state that Grade II carcinoma reveals undifferentiated cells which show distinct nuclei and binucleated forms in transition between basal and squamous cells. They described a mixed basal cell and squamous cell carcinoma occurring in a case of xeroderma pigmentosum and mention that in cutaneous basal cell lesions where metastases occur there usually will be found on careful microscopic study a definite transition to squamous cell epithelium. Such a tumor consisting of basal and squamous cells is referred to by Montgomery [7] as mixed basal-squamous cell carcinoma. This author also stressed that the basal cell epithelioma is not a morphological entity, but may through metamorphosis become a basal squamous cell epithelioma or even a squamous cell epithelioma. It is interesting in this respect to note that Ewing [5] states that squamous epithelium tends to hypertrophy when invaded by alien cells and that hyperkeratosis is common in such instances. Martin and Stewart [8] described carcinomas of the skin which they term spindle cell epidermoid carcinoma because of the peculiar spindle shaped cells present. These tumors most often seemed to be a variety of scar tissue carcinoma. Similar tumors, which reveal histologically malignant squamous and

spindle shaped epithelial cells (transitional cells) are also described in mucous membranes of the lip. Because of the presence of the spindle shaped epithelial cells or transitional cells they are somewhat similar histologically to tumors arising from the esophagus, larynx, lungs, cervix, bladder and urethra. It may be pointed out especially that these authors further state that these tumors sometimes are erroneously referred to as carcinosarcomas. Gordon [9] describes an apparently somewhat similar tumor which he called intra-epidermal epithelioma with epithelioma adenoides cysticum. As this study shows, it seems quite evident that not only in the unusual instance, but in a number of seemingly simple basal cell carcinomas, epithelial structures were encountered which undoubtedly are squamous in character.

There are melanotic tumors on record which histologically resemble carcinoma (melanocarcinoma) and those which resemble sarcomas (melanosarcoma). As pointed out before, it is just the melanotic tumor which seems to be characterized by the morphological variations of its constituents. The first case here reported clearly shows in individual sections both types, sarcoma-like and malignant epithelial-like cells. The primary lesion was a tumor on the lower extremities with early metastases in the groin. The other tumor belonging in this group revealed basal and squamous cell features and characteristic nevus cells. It is known that basal cell tumors may be confused clinically with melanomas. Because of the pigmentation in the former, Mallory [10] thought that they might arise from hair follicles. Eller and Anderson [11] mention basal cell epitheliomas with excessive pigment formation. Goldsmith and Freudenthal [12] relate instances of epithelioma adenoides cysticum with basal cell type of cells, pearl formation and pigmentation. Affleck [13] shows pictures of melanoma with squamous cell hyperplasia, and Becker [14] speaks of melanotic epitheliomas, meaning hyperplasia of the surface epithelium with abnormal evolution of the majority of the malpighian cells which were often enormously hyperpigmented. He also described pigmentation in basal cell carcinomas. Ewing [5] stated that with melanomas the swollen hyperchromic and pigmented epithelium is often difficult to distinguish from tumor cells. He also noted pigmentation in basal cell tumors.

The relevant tumors arising in the oral cavity probably fall in the classification of those named by Quick and Cutler [15] "transi-

tional cell epidermoid carcinoma." These tumors were thought to arise either from transitional epithelium or from squamous epithelium which, in its growth, has lost its adult epithelial characteristics and has assumed anaplastic features. These authors stressed the fact that adult squamous characteristics, such as hornification, spines and pearl formation, are absent. In this series, however, the tumors showed in addition to the cells just described, which were termed transitional cells, foci of squamous cell features such as typical malignant squamous cells, keratinization, and so on.

Summarizing, it may be stated that in a group of various single tumors several distinct cell types were found. Ewing has drawn attention to variations of tumor cells in metastatic lesions and in recurrent tumors. He states that in metastases the original tumor structure might not be recognizable. Epidermoid carcinoma may appear as round or spindle cell growths; adenocarcinoma of the uterus may recur after curettage as epitheliomas, and melanoma may recur as round cell, diffuse and perivascular pigment-free tumors. In this study, however, it is shown that in some original tumors variations of cells of which the tumor consists may occur. The question arises whether these variations of cells constitute evidence of anaplasia of a single tumor and signify different ages of cell groups, whether they can be interpreted as evidence of two tumors growing into each other and forming one tumor, or whether (not necessarily indicating anaplasia) they may indicate what might be called morphological variations of tumor cells, as will be discussed later.

As to the possibility of the various cell groups signifying merely anaplasia, the following may be said: the tumor cells present showed changes in their form and arrangement and revealed atypical and asymmetrical mitoses. Therefore anaplasia, one of the most characteristic features of malignant tumors, most certainly explains some of the cellular variations encountered. As a matter of fact, Chambers [2] and other investigators interested in the grading of malignant tumors of the cervix use some of the variations of tumor cells here described as a basis for their grading. Yet it seems that the findings of islands of certain cell groups, morphologically seemingly entirely different from the rest of the tumor cells, in a single tumor, cannot be explained on the basis of ana-

plasia. Furthermore the presence of squamous and basal cells, both in a single malignant tumor and in obviously benign melanotic tumors, must be explained some other way. The other possibility is that the various cell types in a single tumor might perhaps be explained on the basis of "collision tumors." Collision tumor, according to Meyer,[16] is a tumor consisting of two primary tumors which have invaded each other. If this were true of the tumors in this series it would be expected, in serial sections, to trace both tumors to their separate origins. No such two distinct tumors, however, were found. It also seems unwise to classify these tumors as "combination tumors," which are the result of the growth of two different blastomatous portions derived from one stem cell, as for instance, the Wilm's tumor of the kidney. In this type of tumor one would expect more evidence of mixed tumor characteristics than was actually encountered. "Composition tumors" as explanation can also be ruled out since this term signifies those tumors in which both parenchyma and stroma have become blastomatous. As there is no evidence in this series of an original tumor secondarily having changed its characteristics, it is fairly safe to assume that these tumors cannot be classified as "mutation tumors."

Ewing [5] pointed out that carcinomas may sometimes vary greatly in morphology and may lead to much confusion of nomenclature. Malignant tumors, when altered by inflammation, hemorrhage, adaptation to mechanical environments and interference by surgical procedures, tend to assume indifferent structures in which most of the original features are lost and from which, as Ewing [5] points out, it is usually hazardous to attempt to reach any conclusions regarding histogenesis. Because of this, and because of the recognition of cellular variations in individual tumors, it was thought wise to review critically reported instances of so-called collision and combination tumors. In a later study [17] it is shown that a vast majority of reported carcinosarcomas are apparently single tumors. In some of these cases it seems very likely that reported carcinosarcomas really are squamous cell carcinomas with either basal cell or transitional cell features. This is particularly so in the so-called carcinosarcomas of the mouth, pharynx and esophagus.

Basal cells of the malpighian layer are often regarded as multipotential cells. If this assumption were the correct explanation for

the variety of cells found in these tumors, in other words if basal cells under the influence of tumor forming factors were able to form squamous cells as well as pigment bearing cells, and also adenomatous structures, it would be expected that these various cells would be found in individual tumors regularly or at least much more frequently. However, these variations of tumor cells are by no means regularly encountered but are seen only in the occasional case.

The essence of this investigation is that a detailed study revealed that a number of single tumors not only consist of one type of tumor cells but of two or more morphologically different types of tumor cells. These tumor cells seem to be derived from cells which under normal conditions are found in the location subsequently taken up by the tumor. To trace well defined and differentiated cell groups to certain non-differentiated cells always seems hazardous. On the other hand, when basal cells and squamous cells are present within the same tumor it seems more likely that such a tumor originally started in basal cells and squamous cells. In instances where the tumor consists of glandular structures and squamous cells it seems likely that the tumor originally started in the region where both types of structures were present. It would appear that the findings here presented, namely the presence of various types of tumor cells, indicate that these tumors arose not from individual cells or certain cell groups but rather from one area which *a priori* contained the various cells of which the tumors consist, or derivations of these cells.

The practical application of this study seems clear. As far as grading of malignant tumors is concerned, the presence of the various cell types has been taken into consideration already (Chambers [2]). It is known that transitional cell carcinomas of the cervix, transitional cell carcinomas of the mouth, and basal cell tumors of the skin, and so on, are much more radiosensitive than squamous cell carcinomas. It is also established that the prognosis varies according to the type of cells present in the tumor. The type of radiation as well as the prognosis must be changed if a closer study reveals that what is reported as a one cell type tumor also contains other cell types. Naturally a transitional cell carcinoma of the oral cavity with squamous cell features will respond less readily to radiation than a simple transitional cell carcinoma.

Thus in this laboratory if various cell types are encountered the diagnosis of these tumors always states "predominating squamous cell carcinoma" or "predominating basal cell carcinoma," and so on. Basal cell carcinomas which recur after treatment and transitional cell carcinomas which are not as radiosensitive as others almost invariably reveal the presence of malignant squamous cells.

Summary and Conclusions

A study of miscellaneous carcinomas revealed morphological variations among the individual tumor cells of particular neoplasms. These morphological differences were not those always encountered in malignant tumors, but were often so heterogeneous as to obscure the exact nature of the tumor. They were principally brought about by the presence of seemingly different types of tumor cells in an individual tumor. Thus, basal cells or transitional cells were found in squamous cell carcinomas. The presence of various types of tumor cells in single tumors may be due to a number of factors which are discussed. However, the findings here presented seem to indicate that these tumors arose from one area which *a priori* contained the various cells of which the tumor consists, or derivatives of these cells.

Morphological variations must be taken into consideration in the grading of carcinomas and also in the determination of the radium sensitivity of tumors. The relative radium resistance of some basal cell or transitional cell carcinomas could be ascribed to the presence of many squamous cells in both of these tumors.

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DESCRIPTION OF PLATES

PLATE 123

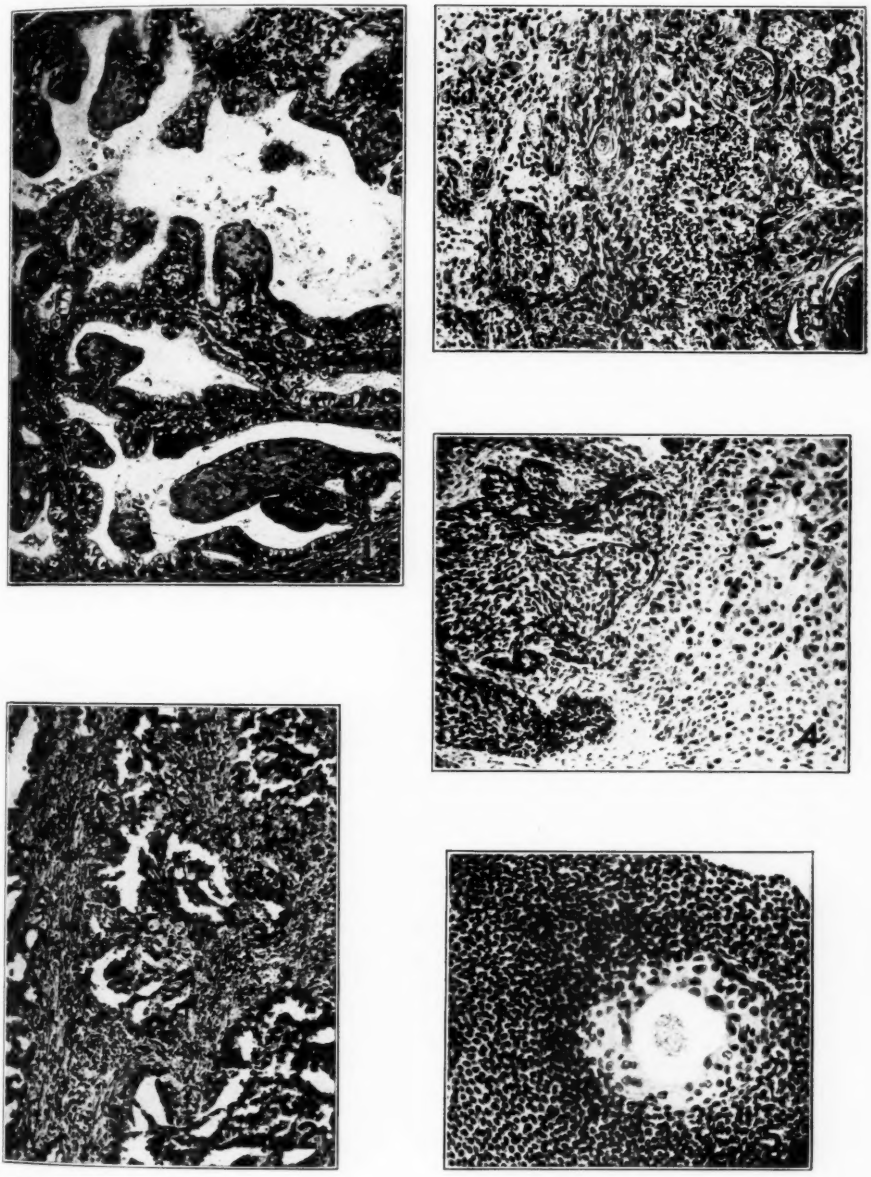
FIG. 1. Uterine tumor. Note the islets of squamous cells in an adenocarcinoma. Hematoxylin-eosin preparation. × 70.

FIG. 2. Adenoacanthoma of the uterus. Note the islet of squamous cell carcinoma in the middle of the picture. Iron hematoxylin-eosin preparation. × 70.

FIG. 3. Carcinoma of the cervix. Note the squamous and transitional cells. Iron hematoxylin-eosin preparation. × 95.

FIG. 4. Carcinoma of the skin. Note the squamous and basal cells. Hematoxylin-eosin preparation. × 80.

FIG. 5. Nevus of the skin. Note the squamous cells in addition to nevus cells. Hematoxylin-eosin preparation. × 135.



Saphir

Morphological Variations of Tumor Cells

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PLATE 124

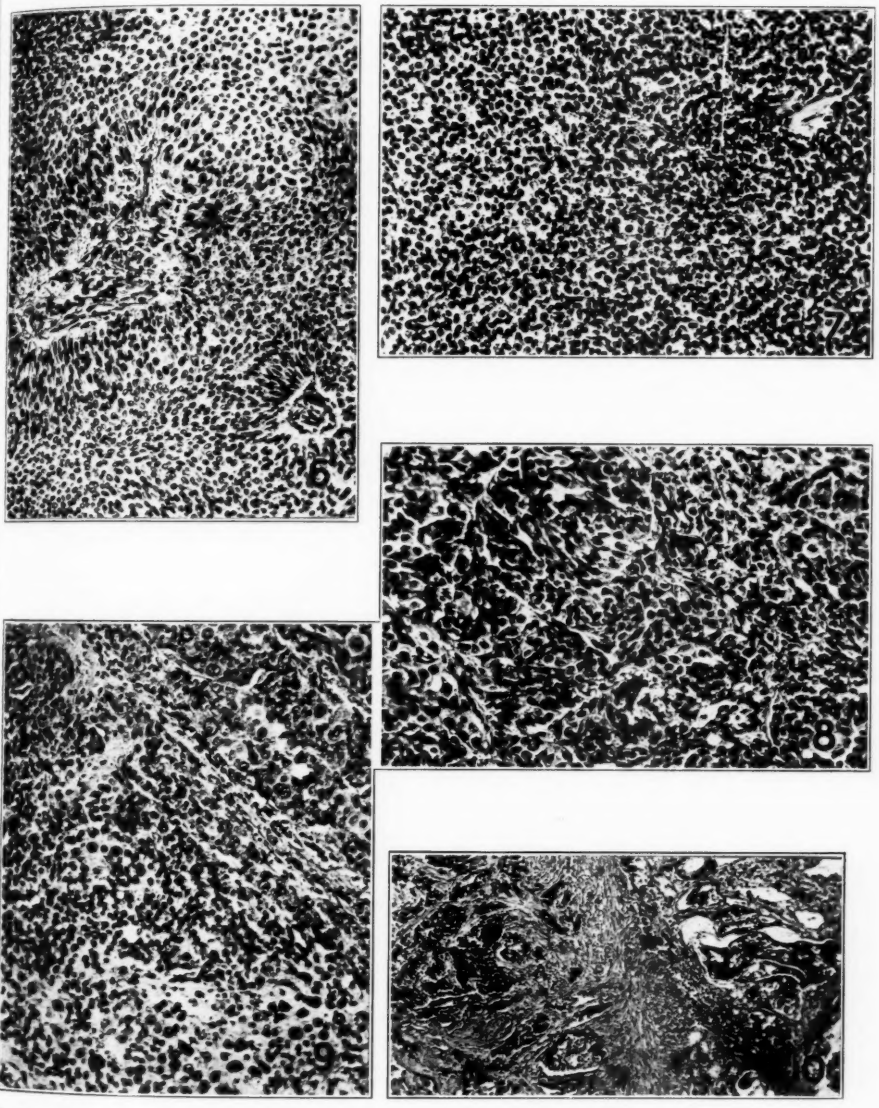
FIG. 6. Nevus of the skin (same tumor as Fig. 5). Note the nevus cells in addition to the basal cell type. Hematoxylin-eosin preparation. × 135.

FIG. 7. Malignant melanotic tumor of the skin. Hematoxylin-eosin preparation. × 100.

FIG. 8. Malignant melanotic tumor of the skin (same tumor as Fig. 7). Hematoxylin-eosin preparation. × 100.

FIG. 9. Carcinoma of the mouth. Note the squamous cells in the right upper part of the field and the anaplastic transitional cells in the left lower portion. Hematoxylin-eosin preparation. × 90.

FIG. 10. Carcinoma of the rectum. Note the adenomatous structures containing mucinous material and the transitional cell type of tumor cells. Hematoxylin-eosin preparation. × 50.



Saphir

Morphological Variations of Tumor Cells

THE MATRIX OF THE EPITHELIAL CELL INCLUSION BODY OF TRACHOMA *

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The epithelial cell inclusion bodies of trachoma were described by Halberstaedter and von Prowazek in 1907.[1] These investigators considered that the inclusions, as seen in Giemsa stained epithelial scrapings, were made up of two components: (1) a bluish staining ground substance or matrix which they called plastin; and (2) a mass of very fine, distinct reddish granules which they termed elementary bodies and regarded as the active agent of the disease. They believed that these elementary bodies multiplied by binary fission and increased at the expense of the ground substance which finally, in the mature inclusion, persisted only in the form of small irregular islands.

The interpretation of the blue substance as a matrix was contested by Lindner,[2] who showed by means of sections and wet-fixed preparations that what often appeared to be amorphous material in dry-fixed preparations was actually a compact mass of well defined, relatively large granular bodies. He studied these bodies, which he termed initial bodies, outside the cell and demonstrated division forms and forms showing transition to the smaller elementary bodies.

This type of morphological variation of the component bodies of cytoplasmic inclusions has since been recognized in two other virus diseases — inclusion blennorrhea [3, 4] and psittacosis.[5, 6] In the latter a dense basophilic staining matrix, masking the component virus granules, was found to be demonstrable in immature inclusions and absent in well developed forms. Ordinary dyes have failed to demonstrate a matrix in the inclusions of inclusion blennorrhea.

In 1936 Rice [7] discovered that the inclusion bodies of trachoma

* This study was made in coöperation with the Health Division, Office of Indian Affairs, Department of the Interior, Washington, D. C.

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and inclusion blennorrhea stain a deep reddish brown with Lugol's solution and from his study he concluded that the inclusion body contained carbohydrate, probably glycogen, diffused through the inclusion to form a matrix. This matrix could be removed by diffusion or by digestion with saliva without affecting the component elementary and initial bodies.

The present study was undertaken in an attempt to confirm the existence of such a matrix. Material was obtained from trachoma cases in Indian children, from epithelial scrapings obtained in the United States Trachoma Hospital at Rolla, Missouri, and from trachoma cases at the Vanderbilt Clinic, New York City.*

The Iodine Staining of Trachoma Inclusion Bodies

Epithelial scrapings from active trachoma cases, both unfixed and fixed with absolute methyl alcohol, were stained with Lugol's solution (unmodified and diluted 1:2 and 1:4) by the method described by Rice. A drop or two of the solution was placed on a dry preparation, covered with a coverslip and examined in the wet state. The inclusion bodies appeared as deep reddish brown masses against a pale yellow background produced by normal epithelial cells, leukocytes and débris (Figs. 1, 2 and 4). Under low power magnification the iodine staining material was sometimes so dense as to appear almost homogeneous, but ordinarily it looked slightly reticular. Under oil immersion (× 1375) this non-homogeneity was seen (Fig. 1) clearly to be due to innumerable minute clear areas, later found to correspond with the granular components of the inclusion, the non-staining elementary and initial bodies. There was excellent contrast between the inclusion mass and the yellow background, particularly in thick smears.

The inclusions could be seen also, though less well, in preparations in which the Lugol's solution had been allowed to dry.

When the iodine stained preparations were later stained with Giemsa and reëxamined, there could be no doubt that the iodine staining material actually represented inclusions. Artifacts sometimes took the iodine stain but were easily differentiable in all cases. There were occasional, black amorphous masses which were believed to be carbonized cell material from the platinum spatula

* I am indebted to Dr. Polk Richards and Dr. C. E. Rice for material used in this report.

used in taking the scrapings. A slight stippling of the cytoplasm of the polymorphonuclear leukocytes occurred, due to the presence of slight amounts of glycogen, but it was never sufficient to cause confusion. Epithelial cells undergoing degeneration sometimes took a diffuse brown stain but it could always be distinguished from the characteristic reddish brown of the inclusion.

For comparison, inclusion-free material was prepared from 10 normal conjunctivae and from 25 cases of bacterial conjunctivitis of different types. No formations in these preparations took the iodine stain characteristic of the inclusion bodies.

A Comparison of Iodine and Giemsa Stained Preparations

Epithelial scrapings from 107 cases of trachoma in Indian children were stained first by the iodine method — methyl alcohol-fixed slides flooded with undiluted Lugol's solution and examined in the wet state under a coverslip. On the mechanical stage and with a magnification of × 250, each slide was examined for iodine staining inclusion bodies. When one was found the cell was marked on the stage so that it could be found again. After complete examination the slide was immersed in distilled water for an hour or more to remove the iodine and was then stained for from ½ to 1 hour in Giemsa's solution, diluted 1 drop to 2 cc. of distilled water. It was then passed through 2 changes of 95 per cent ethyl alcohol and reëxamined. When inclusion bodies were found they were noted on the mechanical stage and a comparison was made with the iodine stain findings.

There was a marked difference in the amount of iodine absorbed by different inclusions, but in general large inclusions stained densely (see Fig. 2), small ones lightly or occasionally not at all: a certain number showed up with the Giemsa stain which had been missed entirely by the iodine stain. Without exception these iodine-negative inclusion bodies were small and made up almost entirely of the large initial body granules. On the other hand, the iodine stain clearly demonstrated inclusions in masses of epithelium which would undoubtedly have been missed in a low power examination of a preparation stained by Giemsa's method alone. The Giemsa stain provides insufficient contrast for the successful demonstration of inclusions in cell masses. Figures 2–5 show the same cells stained first with iodine alone, then also with Giemsa.

The Relation of the Iodine Staining Material to the Elementary and Initial Bodies

There was no evidence obtained to indicate that the iodine staining substance was either identical with the elementary or initial bodies or closely related to them, as would be the capsular material of a bacterium. It could be removed by treatment with saliva without affecting the granules. Further, in 5 of the 107 cases there were numerous free elementary bodies, demonstrable with Giemsa's method, which the iodine stain failed to reveal under oil immersion examination.

The Probable Glycogen Nature of the Iodine-Reacting Substance

Rice was of the opinion that the carbohydrate of the matrix was glycogen because of its microchemical reactions. In the absence of a method for obtaining sufficient amounts of material for direct chemical examination it seemed desirable to compare the microchemical reactions of the substance with those of a known glycogen. Trachoma preparations and impression smears of rabbit's liver known to contain large amounts of glycogen were therefore subjected to a series of tests, conducted under identical conditions for the two substances, with the following results:

(*1*) *Color Reaction:* The iodine reacting substance of the trachoma inclusion body and the liver cell glycogen stained the same shade of reddish brown with Lugol's solution.

(*2*) *Reaction to Heat:* An iodine stained trachoma preparation exhibiting numerous inclusion bodies was flooded with fresh iodine solution, covered with a coverslip and heated over an alcohol lamp. Examined while hot, the inclusions were found to have faded markedly. As the slide cooled the color returned. Liver cell glycogen preparations treated in the same way reacted identically.

(*3*) *Diffusion:* Material from a case of trachoma known to have abundant inclusions was placed in dilute Lugol's solution (1:10) on a slide without being allowed to dry. A considerable number of iodine staining inclusions was noted. The preparation was then placed in a moist chamber to prevent drying and reexamined at intervals of 3 and 4 hours. The color of the inclusions was seen to fade gradually and after 8 hours had disappeared.

The addition of fresh iodine did not restore the color, an indication that the carbohydrate had either been destroyed or dissolved out of the inclusion. The iodine reacting carbohydrate (glycogen) of a liver cell preparation disappeared in the same way under the same conditions.

(*4*) *The Effect of Drying on Diffusion:* Drying effectively prevented diffusion of the carbohydrate from the inclusion bodies. Dried preparations showing typical iodine staining inclusions were placed in distilled water for periods varying from several hours to 2 weeks. The apparent concentration of the carbohydrate, as judged by the intensity of the color reaction when the preparation was restained with iodine, was in no way affected. With one preparation the process of staining and decolorizing was repeated ten times without altering the reaction. Dried liver cell preparations retained their iodine staining carbohydrate under the same conditions.

(*5*) *The Effect of Saliva:* A dry unfixed preparation containing abundant inclusions was treated by dropping a small amount of saliva onto the slide. When it had been incubated for 1 hour, washed in water and restained with iodine, no color-reacting substance could be demonstrated. Liver preparations treated identically also lost their carbohydrate.

The test was repeated with saliva diluted 1:5 with normal saline. Half the diluted saliva was boiled to destroy the ptyalin, half left unheated. The trachoma preparations were placed upright in jars containing the two solutions and incubated for 4 hours. Those that had been treated with heated saliva still gave excellent color reactions; those treated with normal saliva gave none at all. This would indicate that the ptyalin itself was responsible for the disappearance of the carbohydrate. Liver preparations treated in an identical manner gave identical reactions.

(*6*) *Best's Carmine Stain for Glycogen:* Trachoma and rabbit's liver preparations were stained by the method of Best for the demonstration of glycogen. Both the glycogen in the liver cells and the carbohydrate in the inclusion bodies stained well. There was a variation in the degree of staining among the inclusion bodies according to size, the large inclusions staining most intensely.

The results of these several tests would seem to establish the matrix of the trachoma inclusion as glycogen or largely glycogen.

Other Observations on the Matrix

A large number of inclusion bodies stained with Giemsa's method were examined for evidence of a Giemsa staining matrix. None was found. Certain inclusions showed a slight bluish veil over the elementary and initial bodies but this was probably due to the staining of the cytoplasm remaining above and below the inclusion. Preparations made by the wet method of fixation, in which the approximately normal shape of the cell is maintained, were also studied. Stained by Giemsa's method they revealed no staining matrix whatsoever. The elementary and initial bodies were seen to be embedded in what appeared to be a cytoplasmic vacuole which was perfectly transparent as compared to the blue of the unaffected cytoplasm (Fig. 6). Other stains, including neutral red, brilliant cresyl blue and hematoxylin, stained the elementary and initial bodies but not the matrix.

That the matrix has some cohesive properties was indicated by the finding of several intact inclusion bodies which had been extruded from cells. In general, however, there appeared to be wide scattering of the granules after destruction of the cell.

Discussion

The findings in this study corroborate Rice's opinion that the inclusion body of trachoma contains a matrix in which the elementary and initial bodies are embedded and which is composed in large part of a carbohydrate having the microchemical properties of glycogen. The formation of the glycogen may be associated with the conversion of initial bodies into elementary bodies. One preparation which failed to reveal any iodine staining inclusion bodies with Lugol's solution exhibited many initial body inclusions when stained by Giemsa's method. A careful examination disclosed that these inclusions were all in an early stage of development and contained few, if any, elementary bodies.

The chemical composition of the ground substance of cytoplasmic inclusions would seem to vary. The fowl-pox inclusion, for example, is known to contain considerable lipoidal material.[8, 9] The inclusion blennorrhea virus inclusion appears to be identical morphologically with the trachoma inclusion and at the present time is the only other one known to have a carbohydrate matrix.

The inclusion bodies of psittacosis and trachoma have been found by Bedson and the writer to bear a very close resemblance in that the component granules have the same morphological and tinctorial variation and cannot be distinguished from each other when free outside the cells. On the other hand Bedson has been unable to demonstrate a carbohydrate-reacting substance in the psittacosis inclusion; its matrix appears to be associated with the large form of the virus (initial body) and stains densely with Giemsa's solution and other dyes.

Certain workers are of the opinion that the elementary bodies of trachoma should be grouped with the Rickettsiae in view of their large size and parasitic nature and it will be recalled that the minute bodies of psittacosis were at one time described by Lillie [10] under the name *Rickettsia psittaci*. The occurrence in the trachoma inclusion of a stainable matrix, characteristic of viruses but unknown to Rickettsiae, may be of importance in deciding this question. On the other hand, it is true that the elementary bodies of trachoma, inclusion blennorrhea and psittacosis differ sharply in their ease of staining, morphological variation, type of inclusion body and obvious parasitic nature from the typical virus elementary body as represented by the Borrell bodies of fowl-pox and the Paschen bodies of vaccinia.

Summary and Conclusions

The epithelial cell inclusion body of trachoma contains a ground substance or matrix which is composed predominantly of glycogen. This matrix is apparently absent or in low concentration in young inclusions and uniformly present in mature inclusions. Its formation seems to be associated with the change from the large, initial body form of the virus to the small, elementary body form. In view of the ease with which the inclusion bodies may be recognized with low magnification, the iodine stain should prove distinctly valuable as a laboratory test in the diagnosis of trachoma.

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DESCRIPTION OF PLATE

Plate 125

Fig. 1. Epithelial cell inclusion stained with Lugol's solution and showing non-homogeneity of the matrix. × 2000.

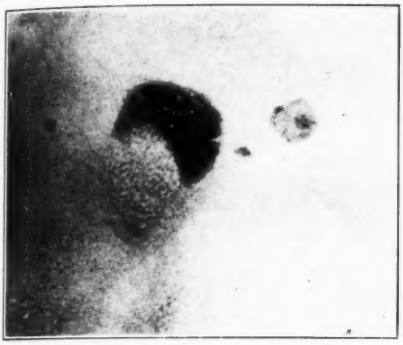
Fig. 2. Large inclusion body occupying the entire cytoplasm of the epithelial cell. Stained with Lugol's solution. × 2000.

Fig. 3. The inclusion body shown in Figure 2 restained by Giemsa's method after removal of the iodine with water. It is seen to be composed of myriads of elementary bodies. × 2000.

Fig. 4. A large inclusion body staining densely with Lugol's solution. × 2000.

Fig. 5. The inclusion body shown in Figure 4 restained by Giemsa's method after removal of the iodine. × 2000.

Fig. 6. A wet-fixed (sublimate-alcohol) preparation stained by Giemsa's method and showing two inclusion bodies. There is no staining of the matrix and the elementary and initial bodies appear to be in a cytoplasmic vacuole. × 2000.



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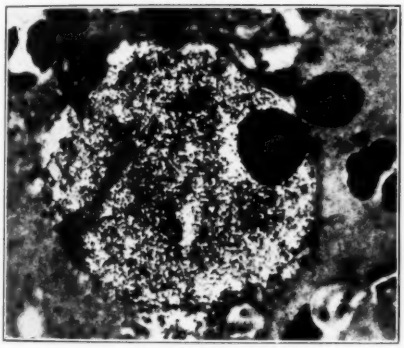

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Thygeson

Epithelial Cell Inclusion Body of Trachoma

UNIV. OF MICH.

THE ABILITY OF LYMPH TO MAINTAIN VIABILITY IN "DEVASCULARIZED" LYMPH NODES *

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In the course of experimental work concerning the physiology of the lymphatic system it occurred to the authors that some light might be shed on the function of lymph if it could be shown that lymph *per se* could keep tissues alive. In the following experiments all vascular connections to the popliteal lymph nodes of dogs were severed, but one or more afferent and one or more efferent lymphatic channels were left intact and it was found that lymph sufficed to maintain viability. These results imply a nutritive function on the part of lymph which, so far as we are aware, has not been demonstrated *in vivo* in mammals.

METHODS

Normal, healthy, adult mongrel dogs maintained on kennel diet and allowed water *ad lib.*, were used as experimental animals. Under ether or nembutal anesthesia the popliteal node was exposed through a linear 4–6 cm. incision. Sterile technique was observed in all operative procedures.

In the *control* group of animals the node was completely excised with a minimum amount of hemorrhage and trauma and replaced in the popliteal space where it was held by suturing the subcutaneous fascia and skin over it. Attempts were made to ligate all severed afferent and efferent lymphatic channels as well as blood vessels.

In the *experimental* group all tissue which could not definitely be identified as lymphatic trunks was severed and these remaining trunks were cleaned as thoroughly as possible of any connective tissue that might carry blood capillaries. With experience one learns to identify lymphatic trunks with certainty by their translucency and the characteristic fashion in which they "bead" below

* Received for publication April 6, 1938.

a temporary obstruction. Especially is the identification of lymphatic trunks certain when a poorly diffusible dye (approximately 1 per cent Chicago blue or pontamine blue dissolved in saline, or preferably dog serum) is injected into the subcutaneous tissues between the toes, and the foot gently massaged. This procedure, which can be carried out before the operation is begun, or by an assistant after the operative field is exposed, almost immediately outlines the popliteal node and its afferent and efferent channels in sharp contrast. These dye injections were made in most instances in the experimental group, but in a number of experiments they were purposely omitted to exclude the possible (antiseptic?) effect of the dye.

At varying intervals after the operation the nodes were removed by biopsy or at autopsy, fixed in Zenker's solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin, the elastic tissue method followed by van Gieson's counterstain, and Foot's modification of Bielschowsky's silver stain. In several instances Gram and methylene blue stains were also made.

In obtaining samples of afferent and efferent lymph for chemical analysis the technique described by Drinker and Field [1] was used. Reducing substance was determined by the Hagedorn-Jensen method [2]; bound carbon dioxide and carbon dioxide combining power by a volumetric method.*

Experimental Observations

The results are summarized in Table I. In the control group, *i.e.* dogs with all blood vessels and lymphatics severed, all 15 nodes rapidly underwent massive necrosis, as illustrated in Figure 1. These necrotic changes, which affected primarily the lymphocytic elements but also involved the reticulum and blood vessels, were maximal by 36 hours, following which the nodes underwent liquefaction, reabsorption and fibrous tissue replacement. It was difficult to follow these changes longer than 3 days as the nodes usually became infected and tended to slough out; in fact, 7 experiments were discarded because the node could not be identified in the depths of the open wound and no remnants were recognizable in histological sections of regional tissue. The almost constant presence of infection in this group at first made us suspect our oper-

* The authors are indebted to Dr. Joseph Victor for carrying out these analyses.

ative technique, but from histological studies of these nodes and especially from bacteriological studies of nodes obtained from normal dogs sacrificed for other purposes, we are convinced that in apparently normal healthy dogs, organisms (*Staphylococcus albus* and *aureus*, hemolytic and non-hemolytic streptococcus) are continually being filtered out of subcutaneous lymph by peripheral lymph nodes. The presence of necrotic tissue, which serves as a favorable medium for the growth of bacteria already present in the nodes, plus the absence of the normal defensive mechanism offered by the lymphatic system, affords an ample explanation for the high incidence of infection in this control group.

In the *experimental* group, *i.e.* dogs with all vascular connections severed but with one or more afferent and one or more effer-

TABLE I

Maintenance of Viability in "Devascularized" Lymph Nodes

Group	Interval between operation and biopsy	Number of nodes	Number of nodes completely necrotic	Number of nodes partially viable	Number of normal nodes	Number of infected nodes
	days					
Control *	1–3	15	15	0	0	15
Experimental †	1–9	20	2	14	4	3

* All vascular and lymphatic connections severed.
† All vascular connections severed, but one or more afferent and one or more efferent lymphatic channels left intact.

ent lymphatic channels remaining intact, the results, though not wholly consistent, are suggestive. Some degree of viability was maintained in 18 of 20 experiments (90 per cent); 4 of the nodes remained grossly and histologically normal (Fig. 2), and only 3 became infected. The explanation for the varying degrees of viability illustrated in Figures 3 and 4 is not clear; possibly mechanical and anatomical factors are responsible, such as kinking, thrombosis and leakage from lymphatic trunks, occlusion of trunks by pressure from without as by edema or fibrin, variation in the number and anatomical arrangement of trunks remaining patent, and variation in the amount of movement of individual dogs and the consequent variation in lymph flow through the nodes. The only evidence we have to support the suspicion that the number of patent channels is important is that when deliberate efforts were made to leave a single afferent and a single efferent channel intact so quantitative chemical studies could be done, all of the nodes

showed some anatomical change but none of them showed the uniform necrosis observed in the control group.

Having found a distinct difference between the experimental and control groups, and having shown that in 4 cases integrity of the node had been maintained, attention was next directed at showing that all the blood supply had been excluded. Three methods were used as follows:

Under nembutal anesthesia the left popliteal node was exposed, all blood vessels severed, and its afferent and efferent lymphatic channels freed for a distance of about 3 cm. so that a rubber tissue dam could be interposed between the node and the underlying tissues. In this way the lymphatic channels entering and emerging from the node could be observed directly at any time during the experiment. The right popliteal node was then excised and placed on the rubber tissue dam beside the experimental node. Both were covered with a thin gauze pad kept moist by a warm saline drip and maintained at a temperature of 35–38 degrees C. by the use of lights. The left hind leg was then secured above the ankle and the foot moved passively with a mechanical device to ensure continuous lymph flow. The animal was maintained under light anesthesia by repeated small subcutaneous injections of nembutal. At 10:30 P.M., 12 hours after the experiment was started, dye was injected into the subcutaneous tissues between the toes of the left hind foot. The afferent channels, the node and the efferent channels became filled with dye almost immediately. Dye injection was repeated just before the experiment was terminated at 8:30 the following morning. By this time gelatinous thrombi had formed in some of the afferent trunks but by massaging upward along the course of the lymphatic trunks from the foot dye could be forced past the node into the efferent channels. At no time during the course of the experiment were any blood capillaries seen coursing along the lymphatic trunks, although these were looked for at frequent intervals. Section of the control right popliteal node showed massive complete necrosis with early liquefaction. The experimental node, however, showed only slight early necrosis in a few places and this may well be related to trauma, "saline extraction," or to the terminal thrombi.

In another experiment the node was enclosed in a rubber dam. This satisfactorily excluded the possibility of granulation tissue

growing through the capsule save along the afferent and efferent lymphatic trunks at the poles of the node which, of course, penetrated the rubber sheath. The experimental node appeared normal when it was excised 7 days later. The opposite popliteal node, which was completely removed and replaced in the popliteal space enclosed in a rubber sheath, showed massive necrosis and infection when it sloughed out 3 days later.

The deprivation of vascular supply was also confirmed by intra-arterial injection of India ink. One to 3 days after isolating the popliteal node on one side, save for its lymphatic connections, the dog was anesthetized and a cannula placed in each femoral artery and tourniquets placed about 5 cm. above and below each "knee." Saline (about 150 cc.) was then perfused through the arteries until the fluid that returned through the cut end of the femoral veins was clear. Full strength Higgins' India ink (about 30 cc.) was then run through the cannulas until the returning fluid was black. Whereas practically every blood vessel in the node on the unoperated side was distended with ink, of the 6 devascularized nodes on which this procedure was tried ink was found in only one small arteriole in 1 node. The necrotic changes in this node were as great as in the other 5 nodes which contained no ink.

Evidence from all three types of experiments indicates that the blood supply to the nodes had been excluded. Any nourishment to the nodes, therefore, must have come by way of the lymphatic trunks or by diffusion through the capsule from the surrounding tissues. That the latter mechanism is totally inadequate to maintain the vitality of the nodes is evident from the uniform necrosis in the control group. Nutritive material must have reached the nodes through the lymphatic channels.

In a limited number of experiments, sugar, bound carbon dioxide, and carbon dioxide combining power determinations were made on afferent and efferent lymph obtained by cannulating lymphatic trunks leading to and from these "devascularized" nodes. The results of 3 experiments are given in Table II.

The data in Table II indicate certain relations between glucose disappearance and decrease in both carbon dioxide and carbon dioxide combining power of lymph during its passage through the lymph nodes. The mean decrease in lymph carbon dioxide and carbon dioxide combining power was 12–13 volumes per cent re-

TABLE II

Chemical Analyses of Lymph Flowing to and from "Devascularized" Lymph Nodes

Number of dog and node	Number of hours after severance of blood vessels	Lymph flow through node †	Sugar		Carbon dioxide				Histology of node
					Bound		Combining power		
			A	E	A	E	A	E	
		cc./hr.	*mg./ 100 cc.*	*mg./ 100 cc.*	*vol./%*	*vol./%*	*vol./%*	*vol./%*	
37–144 (RPN)	2½	0.45	116	59	38	27	61	48	Normal
37–145 (RPN)	3½	1.05	109	66	41	31	61	48	Normal
37–145 (LPN)	24	1.40	146	110	45	30	58	45	Slight necrosis of medulla, follicles viable

A = afferent lymph; E = efferent lymph; RPN = right popliteal node; LPN = left popliteal node.
These figures represent amount of efferent lymph collected from a single trunk.

spectively, which is equivalent to 48–52 mg. of lactic acid. This indicates that under the conditions of this experiment all of the glucose that disappeared can be accounted for by its conversion to lactic acid. With intact blood supply there is no evidence of decrease of glucose from the lymph.[3] Either glucose is supplied to the node directly by the blood or else the loss from the lymph is masked by diffusion replacement from the blood. Under aerobic conditions lymph nodes from mice placed in Ringer's solution produce very little lactic acid from glucose but considerable quantities may be produced anaerobically.[4] Normal lymph nodes also oxidize glucose. Therefore, since under the conditions described above the changes in lymph glucose and lymph carbon dioxide and carbon dioxide combining power are equivalent, they indicate that during the period in which the *lymph gland remains viable its metabolism is mainly anaerobic*. This is not surprising when it is recalled that the rate of oxygen consumption of lymphoid tissue of the mouse is about 1.02 cc. per gm. per hour.[4] If the oxygen content of afferent lymph is equal to that of plasma, then 1 cc. would contain about 5 cmm. of oxygen. Since the rate of lymph flow through a node is about 3 cc. per gm. per hour,* the lymph could supply only about 1 per cent of the oxygen required for respiration.

The obvious step from these *in vivo* experiments, which amount to nothing more than perfusion with lymph, to experiments *in vitro* in which the nodes are perfused with "artificial lymph" and in which there is no question of persistent blood supply, has been taken. The results of early experiments confirm the *in vivo* studies listed in Table II in all respects. These results will be reported later. Suffice it to state here that the chemical studies to date have yielded confirmatory evidence that the blood supply to the nodes has been severed and have indicated that anaerobic glycolysis is one of the metabolic processes effective while they are maintained in a viable state.

Discussion

When it is taken into consideration that lymph was one of the first substances used as a tissue culture medium and that the com-

* This figure is based on an average weight of 1.5 gm. for popliteal lymph nodes from dogs, an average flow of 1.5 cc. per hour per lymphatic trunk,[5] and an average of three afferent trunks.

position of the plasma-Tyrode mixture commonly employed today for tissue culture work closely approximates that of lymph in glucose, electrolyte and protein content, and further that bland infarction of lymph nodes is a rarity, if it ever occurs, the observation that lymph *per se* will maintain the viability of lymph nodes is not surprising. But does this observation give an indication as to the normal function of lymph?

The great bulk of evidence today indicates that lymph, at least in the peripheral portions of the body, is derived from the blood plasma. The rate of lymph formation and the constant presence in the lymph of appreciable quantities of proteins indistinguishable from those in the blood plasma render any other interpretation untenable. Without entering into the controversy as to the identity of tissue fluid and lymph, it is fair to state that all workers are agreed that the fluid which flows through the lymphatic channels represents that portion of the blood plasma filtrate not reabsorbed by the blood capillaries and not utilized by the body cells which are bathed by it before it enters the endothelial lined channels of the lymphatic system to become lymph. That the body cells may add to as well as subtract from the tissue fluid and that all these changes may alter the composition of lymph is also generally recognized. The experimental observations in this paper indicate that this fluid, which has been "rejected" by certain tissues of the body, is still capable of maintaining viability in other tissues. The simplest explanation for this observation is that the excess nutritive material is merely passed on. The magnitude of this "factor of safety," however, is not great * and it is possible that qualitative as well as quantitative factors are involved. How long such nodes can be maintained free from blood supply, whether any morphological changes in the node will occur with time, and

* If the amount of tissue whose lymph drains into the popliteal node of a dog weighing 10 kilos be estimated at 200 gm., and if a pulse rate of 100 and a cardiac output of 30 cc. per beat be assumed, and further if the assumption be made that each gram of tissue in the dog receives the same amount of blood, it can be calculated that the blood flow to the 200 gm. of tissue is 3600 cc. per hour. The total amount of lymph flowing through the node, even under conditions of activity, probably would not exceed 10 cc. per hour; from statistical analysis [6] the actual lymph flow would be nearer 5 cc. per hour, a factor of safety of about 0.2 per cent. On the same admittedly crude basis of computation, the blood flow through the node (assuming a weight of 1.5 gm. for the node) would be 27 cc., or about six times the lymph flow. The actual blood flow through the lymph node is probably much greater.

whether such nodes are capable of hyperplasia or antibody production in response to appropriate stimuli are all questions for future investigation.

Summary and Conclusions

Popliteal lymph nodes of dogs when replaced in the popliteal space after complete severance of all vascular and lymphatic connections rapidly undergo massive necrosis. These nodes usually become infected and may slough out.

When, however, all vascular connections are severed but one or more afferent and one or more efferent lymphatic channels remain intact, infection does not ensue and the nodes remain viable. Chemical analyses on lymph flowing to and from these "devascularized" nodes show a sharp drop in reducing substance, bound carbon dioxide and carbon dioxide combining power in the lymph during its passage through the node, and indicate that anaerobic glycolysis is one of the metabolic processes taking place in the viable node.

These observations imply a nutritive function on the part of lymph which, so far as we can determine, has not been demonstrated *in vivo* in mammals.

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DESCRIPTION OF PLATE

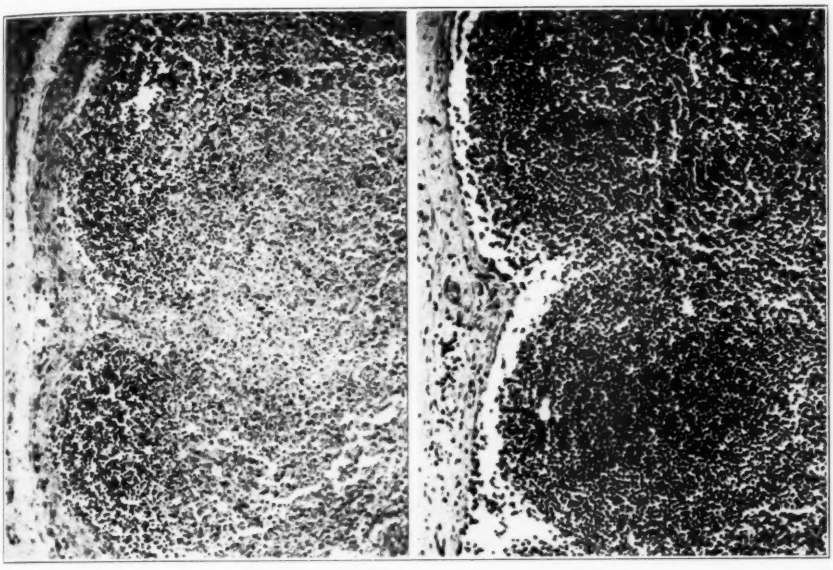
PLATE 126

FIG. 1. Complete necrosis in lymph node 29 hours after severance of all lymphatics and blood vessels.

FIG. 2. Normal structure of node preserved 7 days after severance of blood vessels; lymphatics left intact.

FIG. 3. Partial viability in node 1 day after severance of blood vessels; lymphatics left intact.

FIG. 4. Partial viability in node 2 days after severance of blood vessels; lymphatics left intact.



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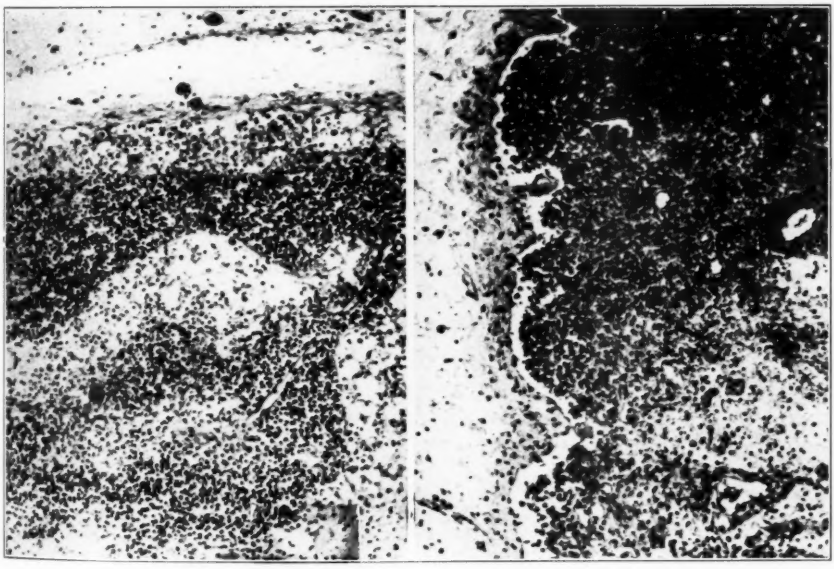

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Holman and Self Viability in "Devascularized" Lymph Nodes

UNIV. OF MICH.

THE RESIDUAL INFECTIVITY OF THE PRIMARY COMPLEX OF TUBERCULOSIS *

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Whether or not the lesions constituting the primary complex of tuberculosis ultimately heal, in the sense that the morbid focus no longer contains virulent organisms of *Mycobacterium tuberculosis*, has been investigated and discussed by many workers since the report of Dejerine [1] in 1884. While morphological criteria frequently suggest that the infective agent in the childhood type of tuberculosis persists for long periods of time, the question cannot always be determined with definiteness unless it can be demonstrated that material from the lesions is infective for guinea pigs. The problem is of clinical significance since the persistence of apparently quiescent or healed tuberculous foci within the body is a definite menace as long as virulent tubercle bacilli remain.

REVIEW OF THE LITERATURE

Dejerine [1] in 1884 was among the first to investigate this problem. He examined caseous and calcified lesions of tuberculosis from 12 subjects who were in the fourth, sixth, seventh and eighth decades of life and failed to find the tubercle bacillus in tubercles that were completely calcified. In tubercles that were completely calcified in the center with traces of caseation at the periphery, the bacilli were found in smears or histological preparations from the peripheral portions but not from the center. Material from 4 of the subjects whose lesions were completely or incompletely calcified was used to inject guinea pigs. The results were all considered negative at the time his paper was published, although the experiments on incompletely calcified nodules were considered as unfinished. Dejerine concluded that when calcification of a lesion is complete the infective agent has disappeared and the lesion may be considered as healed. Kurlow,[2] as a consequence of the results

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obtained following the injection of animals, considered tuberculous lesions that were caseated to be potential sources of reinfection, and that for a lesion to be entirely healed it must be completely calcified. Birch-Hirschfeld [3] studied the incidence of tuberculosis among 826 persons who had died from accidents or from acute diseases and found tuberculous lesions in 171, or 20.7 per cent. Of those showing anatomical evidence of tuberculosis, the lesions in 105, or 61.4 per cent, were considered as healed; lesions in 31, or 18.1 per cent, appeared to be definitely active; and those in 35, or 20.5 per cent, were presumed to be latent or mildly active.

Rabinowitsch [4] tested the infectivity for guinea pigs of calcified tuberculous lymph nodes from 4 adults, 1 of whom had pulmonary tuberculosis. Positive results were obtained in each instance. Weber,[5] by the injection of guinea pigs, studied a series of 39 children less than 15 years of age who had primary tuberculosis of the intestine or mesenteric lymph nodes. Material from 17 of these children whose disease was limited to the mesenteric lymph nodes failed to produce tuberculosis in the experimental animals. The lesions in these 17 cases were caseous and calcified.*

Schmitz [6] injected guinea pigs with material prepared from calcified tuberculous foci from 28 subjects and obtained positive results in 13 instances. The material studied consisted of foci from the lungs and from the tracheobronchial and mesenteric lymph nodes.

Bugge is quoted by Hektoen [7] as having found that 35 per cent of 138 subjects more than 1 year of age who had died from causes other than tuberculosis showed morbid changes that were interpreted as representing healed lesions of tuberculosis.

Opie [8] reported the results of a study designed to determine the incidence and the structural character of lesions of tuberculosis in the lungs of children and adults. Opie's search for lesions was especially thorough, since in addition to the usual dissection methods the different lungs were examined roentgenologically. Of the 93 children examined, lesions of tuberculosis were found in the lungs of 22. Eleven had died of tuberculosis. Of 50 adults examined, tuberculosis was the cause of death in only 3 instances,

* The bovine type of *Mycobacterium tuberculosis* was present in 13 of the 22 cases in Weber's series in which the results were positive. In 7 cases the human type of the organism was demonstrated, and in 2 both the bovine and the human types were present.

yet tuberculous lesions were found in the lungs of all. Morphologically lesions that were firmly calcified in the center and had a capsule of fibrous connective tissue were considered as healed. When young tubercles were present the lesions were considered as active. Opie emphasized the importance of calcification as indicative of the fact that a lesion had healed. He excluded from the group in which the lesions were considered as healed those in which calcification was not complete and those in which caseous lesions were found within the regional lymph nodes. Morphologically Opie classified the lesions in the lungs of the 22 children as follows: healed in 3, caseous and encapsulated in 6, and active in 13. The lesions in the adults were classified as healed in 19, caseous and encapsulated in 25, and active in 6.

In a later study Opie,[9] in collaboration with Aronson, examined material from 97 lungs and 178 lymph nodes from 169 individuals with the childhood type of tuberculosis. The material was used to inject guinea pigs and, when the results obtained from examination of the lungs and lymph nodes were considered together, it was found that living organisms of *Mycobacterium tuberculosis* were present in the different types of lesions as follows: in caseous lesions that were partially fibrotic, 33.3 per cent; in caseous encapsulated lesions, 23.3 per cent; in caseous calcified lesions, 4.4 per cent; and in calcified nodular lesions, 20 per cent.

Opie and Aronson [9] thought it was not improbable that the positive results obtained for guinea pigs that were given injections of material from calcified nodules of the lungs and from the lymph nodes might have resulted from the presence of tubercle bacilli in the tissue surrounding the morbid focus rather than from bacilli within the substance of the focus. To determine the validity of this hypothesis guinea pigs were given injections of fairly large amounts of material prepared from the apices and bases of lungs and from the contiguous lymph nodes of 33 persons. Macroscopically none of the tissues used for injection appeared to be tuberculous although lesions of chronic tuberculosis were present elsewhere in the lung. The results of animal inoculation tests revealed that living organisms of *Mycobacterium tuberculosis* were present in one or the other of the tissues examined in 15 of the 33 cases. The authors concluded, therefore, that the development of tuberculosis in guinea pigs given injections of material from case-

ous encapsulated or calcified nodules of the lung or lymph nodes is due with few exceptions to the presence of tubercle bacilli in the tissues surrounding the focus and not in the focus itself. They believed that, generally speaking, the organism of tuberculosis disappeared from the lesions when calcification became evident.

Griffith [10] in 1929 reported on the types of *Mycobacterium tuberculosis* that had been demonstrated in tuberculous lesions of human beings and included data on a considerable number of cases in which lesions of tuberculosis presumably were present but in which living tubercle bacilli could not be demonstrated by the inoculation of guinea pigs. Of 319 cases investigated there were 47 with gross lesions of tuberculosis in which no living organisms of *Mycobacterium tuberculosis* could be found. Griffith gave a summary of 178 "instances" * in which tissues containing tuberculous lesions had failed to be infective for guinea pigs, although acid-fast forms resembling those of *Mycobacterium tuberculosis* were present. Cultures were attempted in 97 instances, with negative results. Griffith's observations indicated that tuberculous lesions of long duration rarely if ever contain living tubercle bacilli. He found healed lesions in persons ranging in age from 11 months to 70 years and concluded that lesions of human tuberculosis "whether produced by human or by bovine tubercle bacilli and wherever situated, may undergo spontaneous arrest and cure."

Robertson [11] in 1933 reviewed the problem of the persistence of tuberculous infections in human beings and mentioned the relative frequency of cases in which the latent or dormant characteristics of tuberculous infections could be demonstrated morphologically. He reviewed a series of 3306 consecutive autopsies and found evidence of tuberculosis in some form in 2064 (62.43 per cent). In 89 of the cases tuberculosis was either the principal or a contributing cause of death, and in 1725, changes that were considered to represent healed lesions of tuberculosis were found. By morphological criteria such as proliferation of connective tissue, giant cells and accumulations of lymphocytes, Robertson classified the lesions in 134 (4.05 per cent) of the cases as active. He concluded, in part, that tuberculous infections may subside and be regarded as healed, yet remain continuously active, and that tuberculous

* Whether or not the word "instances" represented 178 separate cases was not clear.

lesions, which have apparently healed, may become clinically active after varying intervals.

Hektoen also expressed the opinion that infection with *Mycobacterium tuberculosis* persists for a prolonged period. He maintained that caseous material, such as occurs in tuberculous lesions, is not readily absorbed and that the bacilli may persist even when such lesions are completely encapsulated. He stated that partly calcified sclerotic areas "containing caseous or calcareocaseous foci cannot be regarded as completely healed but rather as having passed into a state of latency." Hektoen considered a tuberculous lesion as healed only when there had been formed a more or less structureless mass, with or without the deposit of calcium and without caseous material being present. He expressed the opinion that caseous material, even of long duration and partially calcified and encapsulated, may contain virulent tubercle bacilli.

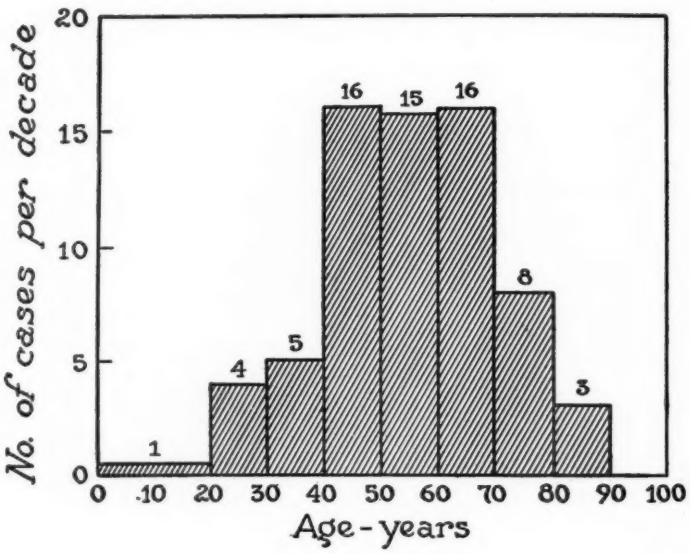
Ghon [12] also believed in the persistence of tuberculous infections. He stated that the primary complex may heal completely, but that in the majority of cases the healing is incomplete, the process passing from a state of activity to one of latency where it may give rise to endogenous reinfection. In discussing the latency of tuberculous infections Calmette [13] mentioned that lymph nodes, especially in the peribronchial and mediastinal regions of adults, may contain small sclerotic foci that are frequently visible only microscopically. He stated that the majority of such lesions still contain virulent organisms of *Mycobacterium tuberculosis* which can be demonstrated by the inoculation of animals.

The literature reviewed therefore indicates that there is no unanimity of opinion concerning the infectivity of chronic lesions of the primary complex of tuberculosis. It is apparent, however, that morphological evidence is not a trustworthy criterion unless the findings are supplemented by the results of animal inoculation tests. Even then there may be difficulty in correlating the pathological changes as viewed microscopically with the results of the tests for virulence, since the identical tissue cannot be utilized for both procedures.

Material and Methods

In contemplating the study which forms the basis of this report, the possibility that lesions of long duration might contain viable

but attenuated or avirulent organisms of *Mycobacterium tuberculosis* was considered. In order to demonstrate acid-fast forms, which might be present but which might not induce demonstrable lesions in guinea pigs, it seemed desirable to inoculate culture mediums with portions of each specimen studied. In other words, an attempt was to be made to determine whether or not living



TEXT-FIG. 1. Age distribution by decades of the 68 human subjects who had chronic primary tuberculosis.

organisms of *Mycobacterium tuberculosis* were present by procedures that had proved dependable in the study of tuberculous materials from many sources.

Material was obtained at autopsy from a total of 68 unembalmed bodies of human beings who had died from causes other than tuberculosis. Evidence of infection with *Mycobacterium tuberculosis* as observed grossly was limited in most instances to well encapsulated caseous or caseocalcareous Ghon tubercles or similarly affected tracheobronchial lymph nodes. The ages of the respective individuals ranged from 7 to 90 years, the largest number having been in the fourth, fifth and sixth decades (Text-Fig. 1).

Twenty-two of the subjects were females, 46 males. There was only 1 negro in the series. The causes of death were as follows: malignant neoplasm, 26 cases; bacterial infection, 15 cases; cardiovascular disease, 7 cases; peptic ulcer, 4 cases; blood dyscrasia, 4 cases; uremia, 3 cases; pulmonary embolism, 3 cases; and miscellaneous causes, 6 cases.

The material obtained for subsequent studies to determine the presence or absence of organisms of *Mycobacterium tuberculosis* consisted of Ghon tubercles, hilar lymph nodes, or both Ghon tubercles and hilar lymph nodes from the same subject. In 5 cases tissues representing so-called apical scars were studied. In these instances, however, a Ghon tubercle or an involved hilar lymph node was also obtained for study. The tuberculous thymus gland was studied in 1 case and in another a tuberculous mesenteric lymph node was utilized. In both cases, however, the extrapulmonary lesions were studied in addition to the lesions that were present in the lungs. In a few cases 2 hilar lymph nodes or 2 Ghon tubercles from the same subject were used. A total of 103 emulsions was prepared from the material secured from these 68 subjects. These were as follows: from Ghon tubercles, 41; from hilar lymph nodes, 55; and from apical scars, thymus and mesenteric lymph node, 7.

Lesions that appeared macroscopically to represent a tuberculous infection were placed in sterile containers immediately after their removal from the body and delivered at once to the laboratory. In most instances a small portion of the lesion was preserved for subsequent histological study. As received at the laboratory the specimens consisted, for the most part, of grossly morbid tissue with a minimal amount of apparently normal tissue at the periphery. In a few instances in which generous amounts of pulmonary tissue containing Ghon tubercles were removed from the body, the lesions were separated from the surrounding tissues of the lung before the nodules of morbid tissue were emulsified. The attempt in every instance was to use for subsequent study only those tissues that constituted the lesion of tuberculosis as the disease is recognized at autopsy, and to avoid if possible the use of surrounding tissues which, while apparently non-tuberculous, might conceivably contain the infective agent in a state of latency.

The respective specimens were prepared for injection into ani-

mals and for the making of cultures by grinding them thoroughly with sterile sand in a small amount of physiological sodium chloride solution. The amount of fluid added to facilitate emulsification of the specimens seldom exceeded 6 to 7 cc. After being emulsified the material was transferred to a test tube which was placed in the refrigerator for from 24 to 48 hours. The supernatant fluid was then used for the making of cultures and for the injection of animals.

Cultures: From the emulsion prepared from each lesion two 1 cc. amounts were used for the making of cultures. For the purpose of eliminating other than organisms of *Mycobacterium tuberculosis* from the material to be cultured the oxalic acid method of Corper and Uyei [14] was followed.* Because of the possible rôle of the bovine type of the tubercle bacillus in infections in human beings and because most strains of the bovine tubercle bacillus when first isolated are non-glycerophilic, it appeared desirable to provide a non-glycerinated as well as a glycerinated medium for the making of cultures. The medium selected was the unheated egg yolk-agar combination described by Herrold.[15] † This was prepared in two forms: one in which glycerin to the amount of 3 per cent was incorporated and one to which no glycerin was added.

From each lesion that was emulsified 2 cc. of the fluid material previously treated with oxalic acid were distributed over the surface of 8 slants of medium, 4 being glycerinated and 4 containing no glycerin. The cultures were incubated at 37.5° C. for from 10 to 12 weeks before the final results were recorded.

Inoculation of Animals: Two guinea pigs were given injections of material from each lesion. Although in the majority of cases only 1 lesion was obtained, in some instances 2 or even 3 separate lesions were used. Consequently in a considerable number of cases 4 or even 6 guinea pigs received injections of the material. The total number of guinea pigs used was 206. All injections were made subcutaneously into the tissues of the wall of the belly.

The dose varied and depended on the amount of material avail-

* The method consists of adding an equal amount of 5 per cent oxalic acid solution to the material to be cultured. The mixture is then placed in the incubator at 37° C. for 30 minutes. It is then diluted with 5 volumes of physiological sodium chloride solution and centrifuged for 30 minutes. The supernatant fluid is then discarded and the precipitate spread over the surface of the culture medium.

† In preparing this medium 15 gm. of agar per liter was used instead of 10 gm. as originally proposed by Herrold.

able. At least 1 cc. was injected into each guinea pig and in many instances 2 cc. or more were given. When guinea pigs died within 21 days after having been given the injections the results were considered as failures. The guinea pigs were killed after the lapse of 8 weeks or longer unless they had died before that time. A large number were permitted to live for 12 or even 14 weeks before being killed for autopsy. The results for 36 guinea pigs that died within 3 weeks after the injection were recorded as failures. In only 3 cases, represented by 8 guinea pigs, did all of the animals die within 3 weeks. The other 28 guinea pigs that died within 3 weeks had been given injections of material from 22 subjects. However, other guinea pigs had been inoculated with portions of the same material from these 22 subjects, and these guinea pigs lived more than 21 days. In the latter instance the survival period was sufficient for lesions of tuberculosis to become evident if the material injected had been infective. Consequently it was possible to draw conclusions as to the infectivity of the material for guinea pigs in all but 3 cases.

The guinea pigs were examined carefully at autopsy and all lesions, even though but slightly suggestive of tuberculous infection, were removed for additional study to determine whether or not *Mycobacterium tuberculosis* was present. The procedure consisted of the making of cultures, the inoculation of additional guinea pigs and the examination of portions of the tissues histologically.

Results of Laboratory Examinations

Positive results were obtained for only 1 of the 68 subjects. In none of the others was the presence of *Mycobacterium tuberculosis* demonstrable by cultural methods or by animal inoculation.

This single subject for whom positive results were obtained was a 54 year old male who had died as a consequence of fracture of the skull. The material obtained for tests for infectivity consisted of 1 encapsulated caseocalcareous Ghon tubercle from each lung. The respective lesions were emulsified separately and each emulsion was used to prepare cultures and to inject guinea pigs. There was no evidence of the presence of viable organisms of *Mycobacterium tuberculosis* in the emulsions made from the lesion of the left lung. From the lesion from the right lung, however, colonies

of acid-fast bacilli were observed after an incubation period of 52 days and lesions of tuberculosis were present at autopsy in both of the guinea pigs that had been given injections of portions of the same emulsion used to make the cultures. One of these guinea pigs died after 32 days. There was an ulcerated area in the skin where the material had been injected. The spleen was slightly swollen and, macroscopically, contained indistinct foci suggestive of a tuberculous infection. Histologically the spleen showed the presence of numerous tubercles in which, by appropriate staining methods, acid-fast bacilli were demonstrated. The other guinea pig died 55 days after having received the inoculum and was found at autopsy to be affected by an extensive tuberculous process. Subsequent studies indicated quite definitely that the organism of *Mycobacterium tuberculosis* demonstrated in the Ghon tubercle from the right lung was of the human type.

Histopathological Characteristics of the Lesions

In 41 cases histological sections from 46 tuberculous foci were made and stained with hematoxylin and eosin. In some cases decalcification of the material was necessary, but otherwise the routine technique was used. In 27 cases sections were not obtained because the tuberculous foci were so small that the entire lesion was used for the injection of guinea pigs and for the inoculation of culture mediums. The lesions were classified into three groups on the basis of their histological appearance. In the first group were placed those lesions that revealed no evidence of activity whatsoever; in the second, those that showed slight or only minimal evidence of activity; and in the third, those lesions that appeared definitely active histologically.

The first group consisted of lesions from 20 subjects, an example of the type of lesion characteristic for this group being shown in Figure 1. It is apparent from this figure that the lesions in this group consisted essentially of a caseous or calcified central area of varying size surrounded by a zone of dense fibrous, and often hyalinized, connective tissue. Giant cells, epithelioid cells, or actively proliferating fibroblasts were not seen in these lesions. An interesting finding in this group was the presence of bone in 5 lesions. In 3 of these the formation of marrow had actually occurred.

The second group of lesions, which were designated as showing slight evidence of activity, was from 22 subjects. Microphotographs of sections from 2 of these lesions from the same subject are shown in Figures 2 and 3. The centers of these lesions, like those of the first group, were always caseous or calcified or both, but the periphery showed, in addition to dense fibrous connective tissue, areas or isolated foci of fibroblasts with or without giant cells. Some of the giant cells were of the Langhans type whereas others were morphologically atypical. In regard to this group it may well be argued that since the lesions were often calcified and always encapsulated by a zone of dense fibrous connective tissue, they should be considered inactive histologically, regardless of peripheral fibroblastic activity. Many workers would probably favor such an interpretation. As a working classification, however, we found this grouping useful even though it may be considered arbitrary.

The third group of lesions was from 4 subjects. These were lesions which we designated as revealing definite histological evidence of tuberculous activity. There was active proliferation of fibroblasts, usually with the association of typical giant cells at the periphery of a caseous area. In all instances there were secondary tubercles beyond the primary focus. These secondary foci had small necrotic centers with surrounding zones of epithelioid cells and lymphocytes. Typical giant cells of the Langhans type were frequent. An example of this type of lesion is shown in Figure 4.

In 5 cases sections of multiple tuberculous foci from the same subject fell into different groups. Thus there were 2 cases in which sections of the hilar nodes revealed inactive lesions, whereas sections from the pulmonary nodules appeared slightly active. In 2 other cases the pulmonary lesions were classified as inactive, whereas the hilar nodes showed slight evidence of activity. In 1 case sections of the hilar nodes revealed minimal evidence of activity, whereas sections of the thymus revealed an inactive lesion.

The lesion in the 1 case that was proved by cultures and by inoculation of guinea pigs to contain virulent tubercle bacilli was found to show but slight evidence of activity histologically and had been placed in the second group. The sections were reexamined carefully in order to determine whether or not they could

be placed in the group designated as active histologically. Careful search, however, convinced us that there was insufficient evidence of activity to place them in this group. A microphotograph of a section of a Ghon tubercle in this case is shown in Figure 5. There was a central area of caseation and a peripheral zone of dense hyalinized fibrous connective tissue. Cholesterol crystal clefts were present, both in the central caseous area and in the fibrous capsule. The continuity of this zone of hyalinized fibrous tissue was broken by foci of actively proliferating fibroblasts, lymphocytes and phagocytes laden with a brownish pigment.

Sections of the hilar node in this case were quite similar to those of the pulmonary nodule. There was a large central area of caseation which showed beginning calcification. There was a peripheral zone of dense hyalinized connective tissue broken here and there by small collections of lymphocytes and fibroblasts. No giant cells or secondary tubercles were present. A finely granular, brownish pigment was present in the fibrous tissue at the periphery of the lesion. Similar pigment also occurred in the phagocytic cells of the non-tuberculous portion of the lymph node. This portion of the section, as well as similar areas in the section of the pulmonary nodule, was found to contain large amounts of silica on examination with the polarizing microscope.

Presence of Silica

On microscopic examination of the sections prepared from the tuberculous lesions we were impressed by the frequent occurrence at the periphery of a brownish granular pigmentation of the connective tissue. The pigment was often associated with dense whorls of collagenous connective tissue (Fig. 6). At other times the pigment was found in phagocytes. In some areas it had the appearance of hemosiderin and in others it was quite evidently carbon pigment. Because of the dense fibrosis associated with the pigment it was thought wise to investigate the silica content by means of the polarizing microscope. Silicious deposits were found in large amounts in all such areas.

In most instances the silica occurred chiefly at the periphery of the lesions. It was thought therefore that the presence of silicious deposits might explain why so many lesions showed histological evidence of slight activity although the presence of active tuber-

culous infection could not be demonstrated by culture or by inoculation of guinea pigs.

In order to establish this point all sections were examined under the polarizing microscope and graded as to silica content on the basis of 0 to 4. It was found that practically all the lesions contained some silica and that the deposits were chiefly at the periphery of the lesions in the fibrous tissue.* However, on comparing the silica content with the presence or absence of histological signs of activity, it was found that no correlation existed. That is, many histologically inactive lesions contained large amounts of silica at the periphery, whereas other lesions classified as active or slightly active contained minimal amounts of silica. None of 4 cases in which the lesions contained secondary tubercles had more than a Grade 1 or Grade 2 deposit of silica.

Comment

The results of this study lend support to the belief that, in the majority of instances, lesions of the primary complex of tuberculosis pass through an involutional process that is unfavorable to the continued viability of *Mycobacterium tuberculosis*. In further support of this interpretation is the fact that though the primary complex is present in 70 to 90 per cent of human beings of all age groups, only a relatively small percentage of the population manifests clinical signs of tuberculosis. Additional evidence for believing that the bacilli responsible for the primary complex of tuberculosis eventually become innocuous is the recent observation of Long and Siebert.[16] They noted that a small number of subjects with calcified lesions of the childhood type of tuberculosis did not react to P.P.D. or to other forms of tuberculin. Similar findings had previously been reported by McPhedran and Opie.[17] They found in a large series of subjects representing 1000 families that calcified tuberculous foci were present in approximately 5 per cent of the individuals who failed to react to the tuberculin test. Whether or not the sensitivity to tuberculin diminishes with advancing age in individuals whose tuberculous infection is limited to the primary complex provides an interesting question. It seems

* In none of these cases was there any doubt as to the original cause of the lesions, that is, they were all grossly and histologically of tuberculous origin. In none of these cases was there any evidence of generalized pulmonary silicosis.

possible, at least theoretically, that sensitivity to tuberculin should diminish as the bacteria responsible for its induction disappear or become non-viable. It has been established experimentally that dead tubercle bacilli may provoke a state of sensitivity to tuberculin. When the lesions are of long duration and contain relatively few bacilli that are non-viable, the ability of the tissues to react to tuberculin may no longer be evident.

The results of our study seem to support those of Griffith who concluded that the chronic lesions of tuberculosis undergo spontaneous arrest and that in such lesions living tubercle bacilli can rarely be demonstrated. Griffith's results as well as our own are definitely at variance with the findings of several other investigators, especially those of Opie and Aronson. The latter obtained positive results by the inoculation of guinea pigs in 30 per cent of the material they examined. However, they did not maintain that the infective agent was present within the substance of the lesions, but rather that it was derived, with few exceptions, from the surrounding lung or lymphoid tissues.

In the material utilized in our work an attempt was made to include in the inoculum only the substances constituting the morbid process. However, the nature of the material precluded the possibility of attaining this objective in all instances. While no considerable amount of the adjacent pulmonary or lymphoid tissue was included in the respective emulsions, at least a small fringe of the surrounding tissues was in many instances incorporated with the morbid tissues from which the inoculums were prepared. While Opie and Aronson's findings possibly provide an explanation for the one positive result we obtained, nevertheless the fact that a small amount of the surrounding, apparently non-tuberculous tissues was included in many of the emulsions we used makes this interpretation unsatisfactory for explaining the negative results in all of the other cases. The only interpretation that seems tenable is that the tissues which failed to produce tuberculosis in guinea pigs or to yield positive cultures of *Mycobacterium tuberculosis*, in our experiment, were devoid of living or virulent forms of this bacterium.

The methods used to demonstrate the tubercle bacillus, if it had been present in a viable virulent state, were those in which reliability has been amply established. To have used cultural pro-

cedures alone would have justified a criticism concerning the adequacy of the methods. However, since for every specimen 2 guinea pigs were used, in addition to 8 tubes of culture mediums, it would seem that had living tubercle bacilli been present they should have become manifest. Their failure to do so properly justifies the conclusion that the material examined did not contain living *Mycobacterium tuberculosis*.

In the attempt to correlate the histopathological findings with the results obtained by the injection of guinea pigs and the inoculation of culture mediums, we were constantly aware of the fact that the material used for the histological study could not be identical with that used for the laboratory tests. As a rule the lesions were cut approximately in half and there was no possible way of ascertaining that the portion used for histological study was the same, either as to morphological characteristics or bacterial content, as the portion used for the injection of guinea pigs and for the making of cultures. Consequently, there is the possibility that in the 4 cases that showed lesions which were active histologically the portion of the nodule from which sections were prepared may have contained viable organisms of *Mycobacterium tuberculosis*, whereas the portion used for the inoculation of culture mediums and for the injection of guinea pigs did not contain the bacilli. Had this latter portion been examined there is the possibility that it would not have appeared active histologically.

Although these possibilities exist they do not constitute a satisfying explanation for the lack of correlation. A hypothesis which may explain the apparent discrepancy between histological evidence of activity as seen in the lesions of the third group and the negative results from the laboratory tests has been advanced by Beitzke.[18] He believed that the secondary, apparently active tubercles at the borders of encapsulated and calcified tuberculous foci are not produced by tubercle bacilli which escape from the primary lesion, but are an allergic response to fresh infection with the infective agent. According to Beitzke, the phenomenon is similar to that which takes place when tuberculin is injected into sensitized animals and sufficient antigen is present in the tissues to provoke a reaction.

Finally, the possible significance of silica remains to be considered in the interpretation of the results. Although no correla-

tion existed between the quantity of silica present and the extent of histological signs of activity, it is nevertheless felt that in many of the lesions which showed histological evidence of activity the causative factor was silica rather than viable tubercle bacilli. This, we believe, is especially true of those lesions that showed minimal evidence of activity, that is, the well encapsulated caseous or calcified nodules of the second group which revealed only slight fibroblastic activity at the periphery and no typical tubercles.

Summary and Conclusions

A study was made to determine the presence of virulent tubercle bacilli in chronic tuberculous lesions of the lungs and contiguous lymph nodes of human beings who had died of causes other than tuberculosis. The lesions were emulsified and material from the respective subjects was used to inoculate culture mediums and to inject guinea pigs. Material from a total of 68 subjects was utilized and negative results were obtained in all but 1 case. The age distribution of the individuals studied ranged from 7 to 90 years, approximately 70 per cent having been in the fourth, fifth and sixth decades of life. Tissues from the lesions of the majority of the subjects were studied microscopically and evidence of activity was apparent in a considerable number. The presence of silica was demonstrated within most of the lesions. The results of this study seem to justify the following conclusions:

1. The lesions of the primary complex of tuberculosis, when definitely encapsulated and sclerotic, or caseous or caseocalcerous, seldom contained viable or virulent organisms of *Mycobacterium tuberculosis*.

2. The presence or absence of viable or virulent organisms of *Mycobacterium tuberculosis* in the lesions of the primary complex of tuberculosis cannot be established by morphological appearances alone.

3. The data suggest that in adults endogenous reinfection is unlikely to occur from lesions of the primary complex.

4. The presence of silica in varying amounts is a fairly constant finding in lesions of the primary complex of tuberculosis. In the absence of demonstrable viable tubercle bacilli in the lesions it is suggested that histological signs of activity are possibly due to silica.

NOTE: We wish to acknowledge the assistance of Drs. Lall G. Montgomery, D. C. Beaver and J. C. Henthorne, who aided materially in collecting the specimens utilized in this study.

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DESCRIPTION OF PLATES

Plate 127

Fig. 1. Ghon tubercle with no histological evidence of activity. × 85.

Fig. 2. Portion of periphery of a Ghon tubercle showing fibroblasts, mononuclear cells and lymphocytes which suggest activity of a mild degree. × 130.

Fig. 3. Portion of a hilar lymph node from the same subject. One giant cell of the Langhans' type is present in the midst of hyalinized connective tissue. × 130.

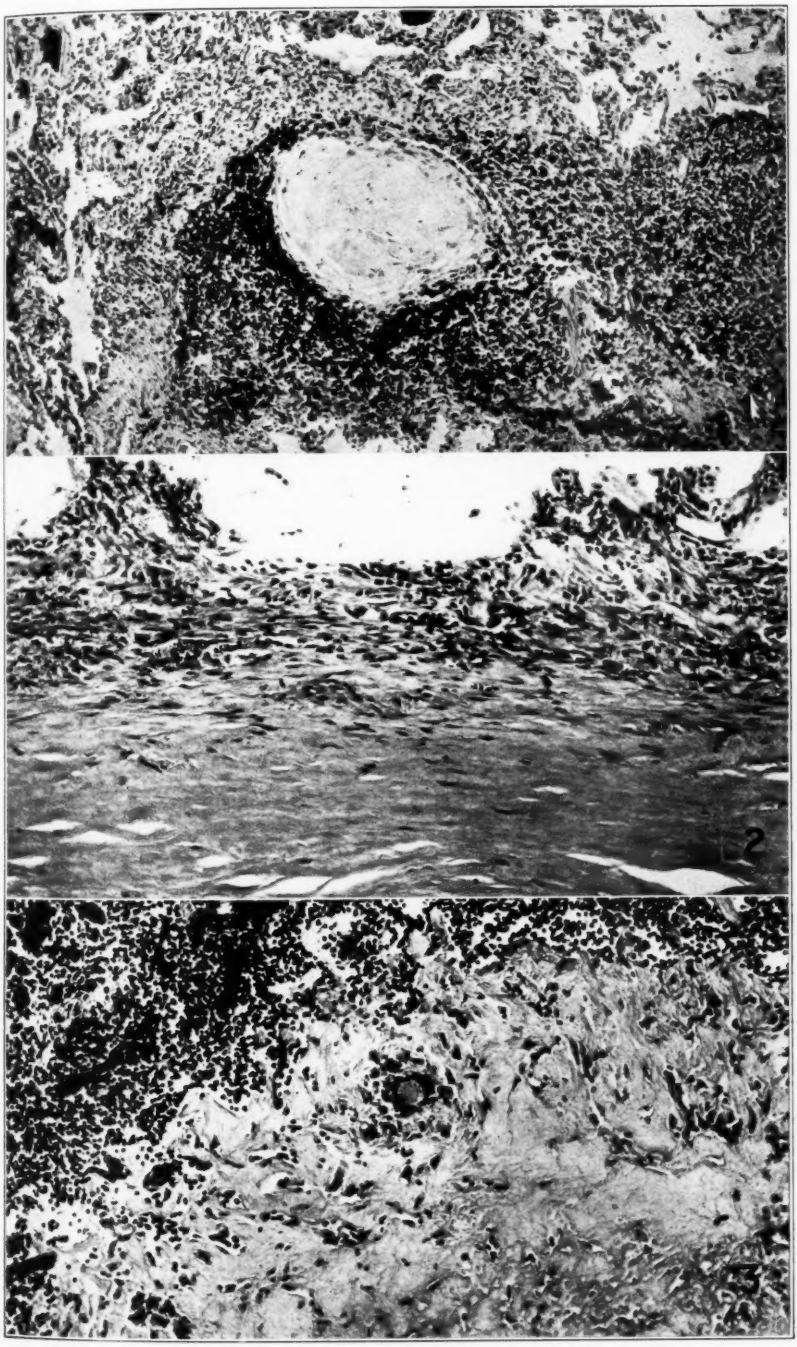

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PLATE 128

FIG. 4. Conglomerate tubercle with several giant cells in a hilar lymph node. × 100.

FIG. 5. Portion of Ghon tubercle in which virulent tubercle bacilli were demonstrated. × 130.

FIG. 6. Hilar lymph node showing evidence of activity of the connective tissue elements. Large amounts of silica were observed in this lesion by polarized light. × 120.

Feldman and Baggenstoss

Infectivity of Primary Complex of Tuberculosis

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THE STAINING OF ACID-FAST BACILLI IN PARAFFIN SECTIONS *

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General Considerations

Ficker [1] in 1929 reviewed and collected from the literature 95 articles dealing with methods of staining acid-fast bacilli, chiefly in smears of cultures and sputums, parenthetically stating that the best review of the literature listed 716 articles on the subject. The number of methods directed at the staining of bacilli in sections is much smaller than this, but still so large that it would be nearly impossible to undertake to examine all the variations critically by comparing them. It would seem probable that there is no single method of staining acid-fast bacilli in tissues capable of meeting all demands and individual tastes, and it is our purpose here not to offer a method calculated to satisfy all difficulties but to consider the various factors involved and the difficulties that the problem presents. No effort is made to review the literature on the subject adequately, or to claim originality for many of the observations made. It would be difficult to introduce a chemical or dye substance into the staining process which has not long since been tried and found useful or discarded.

Koch,[2] in his original paper (1882) on the discovery of the tubercle bacillus, described the staining of bacilli in tissues with alkaline methylene blue, counterstained with vesuvin (Bismarck brown). Ehrlich and he in the same year employed an aniline water methyl violet or fuchsin; Ziehl [3] substituted phenol for the aniline and Neelsen in 1885 devised the general formula for carbol fuchsin that is largely used today. Much [4] in 1907, by modifications of Gram's method (1884), demonstrated granular forms not stained by the Ziehl method. In the innumerable subsequent methods only the dyes of the triphenylmethane group have been much used, pararosaniline and its derivatives. The greater bril-

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liancy of the bacilli stained with fuchsin has apparently proved on the whole superior to the darker color of methyl and crystal violet, judging from the greater popularity of the former.

Fixatives

The original observation that *alcohol is the fixative of first choice* for purposes of staining acid-fast bacilli in sections has not been located, but the many authors who have compared the virtues of different fixatives invariably agree upon it. The fact cannot be exaggerated; in tissues fixed in alcohol acid-fast bacilli are stained rapidly and resistantly. The importance of alcohol fixation is much greater when one is dealing with acid-fast organisms difficult to stain in tissues, such as the human lepra bacillus. Alcohol is not, however, a generally satisfactory tissue fixative; it produces much shrinkage around the margins of the piece of tissue. With larger fragments this is not of as great a disadvantage as when quite small pieces of tissue are to be sectioned. The addition of 10 per cent formaldehyde to the alcohol improves the fixation without affecting the staining of the bacilli. Klingmüller [5] recommends the use of 5 per cent mercuric chloride in alcohol as a fixative. The use of increasing strengths of alcohol avoids part but not all of the shrinkage. In ordinary pathological work the foresighted use of alcohol as a fixative is a most uncommon practice, but in experimental work the disadvantages of alcohol will often be found overshadowed by its virtues.

The various fixing fluids containing mixtures of potassium dichromate and formaldehyde are combinations of an oxidizing and a reducing agent, which react to oxidize formaldehyde and to reduce potassium dichromate with the deposition of chromium salts in the tissues. Joannović [6] states that formaldehyde alone is a poor fixative for acid-fast bacilli, though others disagree. It is probable that formaldehyde is fairly effective in fixing the lipoids of the acid-fast bacilli. Our experiences with decalcifying fluids (such as 5 per cent nitric acid) indicate that these are most deleterious to the achievement of acid-fast stains. This suggests that the formic acid formed in Orth's or Zenker's fluid with formaldehyde might exert a harmful effect. Actually the formic acid formed in these fluids is probably largely decomposed. If tissues are left standing in formaldehyde alone, however, much formic acid is formed, and

tissues preserved in formaldehyde for long periods are nearly useless for acid-fast stains. On the other hand, it seems that *brief* fixation in neutral formaldehyde, while not ideal, may be satisfactory.

That chromium salts are deposited in the lipoids of acid-fast bacilli can be directly observed by adding a solution of potassium dichromate to a culture of tubercle bacilli. This does not affect their tinctorial properties, nor is their acid-fastness lessened by soaking in alcohol and chloroform. But when the culture, treated 30 minutes with potassium dichromate, dehydrated in alcohol and chloroform, is treated with melted paraffin at 55–60° C., the acid-fast properties of the bacilli begin to weaken. There is much confusion concerning the exact effects of more prolonged treatment of sections in chloroform, xylol and paraffin, but it is quite clear that ill effects are particularly observed in tissues fixed in potassium dichromate. Joannović and Klingmüller both emphasize the fact that the disadvantages of fixation in Orth's, Müller's, or Zenker's fluid can largely or completely be overcome by thoroughly washing the excess fixing fluids out of the tissue in running water. This is undoubtedly of the greatest importance, but it is probably not true that tissues so treated ever measure up to tissues fixed in alcohol, for purposes of staining acid-fast bacilli. We have never used Zenker's fluid containing acetic acid, but it appears that the mercuric chloride in the solution does not affect the staining of acid-fast bacilli, although its action as a general fixative is useful.

The deleterious effect of autolysis on acid-fast bacilli is probably measurable. In thick blocks of tissue in which the central part is not fixed, or poorly fixed, the difference in the tinctorial properties of bacilli in the fixed and unfixed parts of the block is noticeable. Successful staining of acid-fast bacilli in sections demands careful attention to the process of fixation.

Clearing of Mercury Deposits from Tissues

The classical method of removing the mercury deposits from the tissues with Lugol's iodine-potassium iodide mixture is wholly satisfactory, but, unless completely removed, traces remaining are likely to cause unsightly insoluble precipitates of fuchsin in the section in their neighborhood. Any acid-fast organisms nearby such deposits will be most brilliantly stained. Removal of the

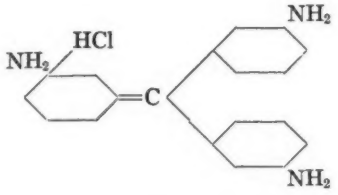
iodine by alcohol is often a slow process; conversion by sodium thiosulphate into sodium iodide is very rapid, but tends to leave water-insoluble deposits which will precipitate fuchsin. Traces of iodine left in the section may not interfere with the staining process, but it seems best to treat the section with both alcohol and sodium thiosulphate (in this order) to avoid all difficulties.

Oxidation and Reduction

It has been our experience, especially with tissues fixed in fluids containing potassium dichromate, that treatment with potassium permanganate 0.25–1 per cent for a few minutes, followed by oxalic acid, definitely improves the staining of acid-fast bacilli, although it will by no means obviate the disadvantages of these fluids. This process tends to loosen sections from the slides and to cause shrinkage. In some instances overtreatment may loosen lepra bacilli from the cells, extracting them from the section. Gömöri [7] advises the use of a gelatin-glycerin mixture for affixing sections to slides, followed by drying in formaldehyde fumes. We have, however, encountered no difficulties if a glycerin-albumin mixture is used, prepared from the whites of fresh eggs.

Fuchsin

Basic fuchsin, magenta and aniline red are names under which mixtures of pararosaniline and its three methylated compounds are sold; most commonly it is a mixture of pararosaniline and rosaniline.



The above formula is that usually given for pararosaniline chloride. It has an unsaturated carbon atom in each phenyl group, and one to three methyl groups may be substituted at these points, yielding four possible compounds. In practice the methylated dyes are prepared by oxidizing equivalent mixtures of aniline and toluidine, with the aid of a metallic catalyst, and are purified by treating with an acid, yielding salts of rosaniline and its derivatives.

Pararosaniline, rosaniline (with one methyl group), and the trimethylated compound (new fuchsin) are obtainable in pure form.

By treating pararosaniline with methyl or ethyl iodide, methyl or ethyl groups replace the hydrogen atoms in the amine groups, anywhere from three to six in number. The lower triethylated compound largely composes the dye known as dahlia or Hoffmann's violet. Mixtures of the methylated compounds are known as methyl violet. The pure hexamethyl pararosaniline is crystal violet.

In our experience the trimethylated pararosaniline (with the methyl group attached directly to the phenol ring), triaminotritolyl methane chloride, fuchsin NB, magenta III, isorubin, or, most commonly, *new fuchsin*, possesses much greater powers of staining acid-fast bacilli than do the pure simpler compounds, or any of a number of samples of basic fuchsin we have employed. It possesses a somewhat darker, less red and more magenta tint than does rosaniline, but the dyed bacilli display greater sharpness of outline and deeper staining. Irrespective of the fixative employed, when adjacent sections are stained for varying periods of time for purposes of comparison, in new fuchsin and rosaniline, it has always been observed that the new fuchsin acts more rapidly, giving a more uniform result and deeper stain. It is only when the time of staining (at room temperature) is prolonged that the effect of rosaniline approaches that of new fuchsin. It seems that new fuchsin should wholly displace the other fuchsins as a dye for staining acid-fast bacilli in tissues.

Carbol Fuchsin

1. Acid-fast bacilli can be effectively stained in a simple aqueous solution of fuchsin at room temperature under favorable conditions of fixation; many hours may be required.

2. A saturated solution of fuchsin in 95 per cent alcohol leaves the bacilli neither acid- nor alcohol-fast after several days' staining.

3. Acid-fastness and alcohol-fastness are wholly relative terms. The best stained acid-fast bacilli are eventually wholly decolorized by alcohol (2–3 days). Strong acids alone accomplish the same effect, possibly with eventual decomposition of the fuchsin, but in a wholly different manner.

4. The acid-fast staining reaction is thus a reversible one. It

takes place poorly or not at all in solutions of fuchsin in solvents in which the fuchsin is readily soluble. Saturated solutions of fuchsin in aniline or phenol, having a dye content many times that of Ziehl-Neelsen's carbol fuchsin, produce indifferent results.

5. The following three solutions have been compared:

A.	New fuchsin	0.25 gm.
	Phenol crystals	5 gm.
	Water, to make	100 cc.
B.	New fuchsin	0.25 gm.
	Alcohol	10 cc.
	Water, to make	100 cc.
C.	New fuchsin	0.25 gm.
	Phenol crystals	5 gm.
	Alcohol	10 cc.
	Water, to make	100 cc.

Only the third solution (C) is a highly effective staining solution. Both the 10 per cent alcohol and the 5 per cent phenol are essential.

6. If the section is treated before staining in:

Phenol crystals	5 gm.
Alcohol	10 cc.
Water, to make	100 cc.

the staining takes place much more rapidly. Omission of either ingredient from the solution largely nullifies the effect. The alcohol is as important as the phenol for the enhancement of the staining; its rôle is more than that of a solvent for the fuchsin.

7. Many substances have been substituted for the aniline or phenol in the preparation of fuchsin, or added to the mixture — boric acid, petroleum ether, aniline black,[8, 9, 10] glycerin,[11] and salt.[12] We have ourselves tried in addition many other compounds, phenols, amines, salts, and so on. Nothing has been found giving results superior to phenol; many substances, however, are more or less effective, particularly when they are present in concentrations close to the point at which fuchsin is precipitated by them.

8. The phenol in carbol fuchsin apparently plays a complicated rôle; it appears to combine with the fuchsin and with the acid-fast material in the bacilli. Its rôle in dissolving the fuchsin is less certain.

Use of the Dye

One of the most variable factors in all modifications of methods employing fuchsin has been the length of time and amount of heat required to stain the bacilli. There are three common methods employed: (1) staining at room temperature; (2) staining immersed in the dye at elevated temperatures, 37–70° C.; and (3) staining by steaming the fuchsin on the slide. Each may be said to have its virtues. The first is the simplest and slowest. The second is the most effective and most injurious to the tissues. The great virtue of the third is its rapidity. For comparing the effectiveness of different dyestuffs or solutions we have used only the first.

Time and heat play the same rôle in the mechanism of the acid-fast reaction. Results are obtainable by staining at room temperature which are wholly the equal of those obtained by using heat. The lower the temperature, the longer will be the time required. The length of time, or the amount of heat required, is also dependent on the method of fixation of the tissue. Steaming the slide is probably the least reliable method in unpracticed hands; it is inconvenient for staining large numbers of sections at one time.

It may seem an unnecessary tautologism to emphasize that the important point of any method is to obtain adequate staining of the organism. The process should be carried to the point beyond which there is no further accumulation of the dye in the bacilli. Much examination of incompletely stained bacilli leads us to believe that the staining takes place in a peculiar but orderly fashion, because the material in the organism giving the acid-fast reaction is by no means evenly distributed through the bacilli, but is concentrated at a few places. Incompletely stained bacilli are usually not weakly and evenly stained but often have a beaded or granular appearance, even to appearing as masses of cocci. With more intensive staining they appear as solid, evenly stained shafts. When acid-fast bacilli occurring in numbers show this granular effect it is almost certain that the tinctorial effect is not maximal. We have no doubt that much of the effect of Much's staining methods is due to their inadequacy completely to stain the acid-fast matter in the bacilli, paradoxically combined with the superiority of crystal violet to most basic fuchsins in staining acid-fast organisms.[13]

The commonly seen beaded appearance has often been considered possibly to be somewhat of an artefact. Cowdry and Heimburger [14] reported that by using Gersh's technique of freezing the tissue in liquid air and dehydrating the solidified tissue *in vacuo*, this beaded appearance was not seen when the tissues were stained with fuchsin.

What has been said above of heat concerns its effect on the staining process, that is, the chemical reaction. But there is a separate effect of heat involved necessarily in staining with the aid of heat, namely, the effect on the carbol fuchsin itself. This results in the greater and more brilliant redness acid-fast substances display when stained by fuchsin which has previously been heated to the boiling point. The greater brilliance is obtained equally well when the bacilli are stained cold with a previously heated solution, as when the bacilli are stained by an ordinary solution with the aid of heat. On cooling a heated carbol fuchsin a good deal of the dye is precipitated, which vitiates the strength of the solution. Many writers caution against the use of heat in preparing the solution.

Thus, heating, which produces greater brilliance and more rapid staining, is a mixed blessing. The disadvantages are waste of dye, greater injury to sections, and a distinct tendency to alter the form and outline of the bacilli, making them appear shorter and plumper, or, in leprosy, causing the masses of bacilli (globi) to fuse together and lose individual distinctness.

The amount of fuchsin called for by different methods varies from 0.3–1 gm. per 100 cc. We have seen very few samples of basic fuchsin that are sufficiently soluble to yield the latter strength. Conn [15] gives the solubility of new fuchsin in alcohol as 3.2 per cent and in water as 1.13 per cent, but we have not seen samples possessing any such great water solubility as this.

When solutions of carbol fuchsin are prepared from a saturated alcoholic solution and 5 per cent phenol water, the resulting strength will be only that of 1/10 the solubility of the dye in alcohol. With pararosaniline this would be theoretically 0.59 per cent, with rosaniline 0.82 per cent, and with new fuchsin 0.32 per cent. Grübler's "fuchsin for bacilli" is the most soluble basic fuchsin we have seen, 1 per cent carbolated solutions being preparable from it, but the dye content appears to be well below that of a pure

rosaniline. Few of the American products yield more than a 0.3 per cent solution, but they appear to be as effective as Grübler's.

Fuchsin in carbolated solutions slowly enters into a partly colloidal state (especially if tap water is used) which probably lowers its effective strength. Manifestly, the effectiveness of any solution is directly proportionate to the dye content, but the use of saturated solutions predisposes to precipitation. A saturated carbol new fuchsin contains approximately 0.9 per cent dye. New fuchsin is somewhat more soluble in methyl than ethyl alcohol, and we have used the following solution which has an effective dye content of 1 per cent:

New fuchsin	1 gm.
Phenol crystals	5 gm.
Methyl alcohol	10 cc.
Distilled water, to make	100 cc.

The dye is wholly dissolved in the mixture of alcohol and phenol, and the distilled water is added, not too rapidly. The solution should be clear and not require filtration. Fuchsin is so readily precipitated by a wide variety of substances that to maintain the full strength distilled water of high purity must be used. The solution preserves indefinitely, but it should be remembered that evaporation of 5 per cent by volume, which will largely be alcohol, will precipitate dye to a much greater proportion. Solutions left in Coplin jars lose strength rapidly.

Decolorization and Differentiation

The second variable in many modifications of acid-fast stains lies in the manner of decolorization. Alcohol is an essential ingredient, but the number of acids that has been suggested is very large.

There is probably nothing more futile than trying to accomplish decolorization of the tissues without that of the bacilli by employing weak acids or weak solutions of strong acids when the bacilli are not too well stained. It can be done at times but not with consistency. Not all mineral acids exhibit the same power of extracting the fuchsin. Hydrochloric acid is most effective, nitric acid less so, and sulphuric still less; but there is no acid highly ionized which is wholly ineffective and there is really not much choice be-

tween several. Fuchsin undergoes a change of color in acid from red to yellow but combined with tissues it may be blackened. With sulphuric acid some of the dye is sulphonated to form acid fuchsin so that the solution regains the red color. In alcoholic solution the color change is different. In weak acid alcohol the fuchsin becomes first a darker bluish red, gradually turning to a deep bluish violet and only turning yellow when the proportion of acid is much higher than ordinarily used. An acid alcohol which turns the dissolved fuchsin yellow also decolorizes the bacilli rapidly.

The classical 1–5 per cent hydrochloric acid in alcohol (70–95 per cent) is wholly satisfactory. The higher percentage of acid leaves the acid alcohol blue, the weaker leaves it red. The lower strength has nearly the same decolorizing effect as the higher, and is preferred. When the acid alcohol leaves an undesirable amount of fuchsin in the sections it can rarely be further decolorized by a stronger acid.

There are various methods that use the acid and alcohol separately. Much stronger acids are employed. The same strengths in alcohol would decolorize the bacilli. In other words, the acid is much more effective in alcoholic than in aqueous solution. The problem in staining acid-fast bacilli in sections rarely consists of a test of the acid-resistant properties of the organism, and such procedures seem to possess no advantages.

The use of picric acid has been made the *punctum saliens* of many methods and is an alluring medium, as we have found. Perhaps the picric acid, which may be effectively substituted for iodine in Gram's stain, behaves in the same way toward fuchsin. But it is not to be recommended for sections, for, unless the picric acid is removed by acid alcohol, the bacilli fade.

The pale pink residual background (sometimes fairly deep) may be treated in three ways: (1) it may be allowed to remain; (2) it may be masked by a heavier diffuse blue counterstain; and (3) it may be removed. The amount of residual fuchsin in the section depends on the time and heat used in the staining process, being greater with prolonged staining and heaviest in tissues subjected to long periods of fixation in heavy metals. In tissues fixed in formaldehyde the red blood corpuscles retain much dye. It cannot be removed by acids or alkalis. The difficulty with obliterating a heavy residue by a heavy counterstain (Delaney [16]) lies in

the fact that the heavy counterstain necessitates a very brilliant lighting, and the bacilli may take some of the counterstain. Under some circumstances this may be necessary.

When the residual color is quite light it may form a fairly satisfactory background of itself, not in any way confusing the brilliant color of the bacilli. In some tissues, such as the skin, the effect is often excellent when a simple nuclear counterstain, such as hematoxylin, is employed.

The removal of the residual fuchsin can be accomplished in several ways: by bleaching with potassium permanganate and oxalic acid, or by treatment with any of several substances which decolorize fuchsin. The coloring of the bacilli is not wholly resistant to any of these procedures, but brief decolorization will often serve to remove much of the residual dye from the tissues without seriously affecting the color of the bacilli. We have used with success a 5 per cent aqueous solution of potassium cyanide. Other substances, such as sodium metabisulphite, trichloracetaldehyde, aqueous benzaldehyde, aniline and sodium sulphite, have been recommended. The difficulty with the sulphites, and other substances which decolorize fuchsin rapidly, lies in the fact that the reaction of decolorization is reversible. Extraction of the dye from the tissues is slower and is inevitably accompanied by the removal of some from the bacilli. Such procedures must be used with caution, but they are at times very helpful.

The Counterstain

The choice of the counterstain permits of no dogmatic assertion as to the superiority of one or another. Nearly every blue dye has at one time been recommended, and individual taste is more than likely to be at the basis of many proposals. We have tried a number of them, particularly alum hematoxylin, and the methylene blue complexes, but satisfactory results may be obtained with polychrome methylene blue, methyl blue and several green dyes. A thorough consideration of all the possibilities would be endless.

In general, it seems that the depth of the counterstain required varies according to the residual fuchsin in the tissues. We must confess a distaste for this residual dye and a necessity of employing a heavy counterstain to counteract it. In rodents particularly, the use of any of the methylene blue complexes dyes the granules

of the tissue mast cells intensely, which is a virtue in view of their often confusing acid-fastness. Very beautiful results are obtainable with these dyes.

Probably the simplest counterstain is methylene blue in a 0.1–1 per cent solution rendered very slightly alkaline with a few drops of ammonia. Only a few seconds are required and the depth is readily controlled by differentiation in a very weak acid alcohol. For color contrast with the red bacilli the color of the methylene blue dyes is probably superior to all others. For alcohol-fixed material it seems to give better results (or, rather, is easier to handle) than azure A, or azure II.

Hematoxylin (Erhlich's, Harris', or Delafield's alum hematoxylin) gives a sharper nuclear stain, more permanent than that of methylene blue. In exudative or necrotic lesions in which many cells are undergoing destruction it may be essential. Used alone in some tissues, particularly those with a surface epithelium, it may be adequate, but overstaining is needed to obtain a good contrasting color in structures other than nuclei. Bluing the hematoxylin is best done in running tap water for 20 minutes or more or in lithium carbonate. Hematoxylin is best used simply as a nuclear stain, with the use of another dye (methylene blue) as a general counterstain. We are not certain that it may affect the permanency of the acid-fast stain adversely.

It is possible, as has been recommended, to use the counterstain before the fuchsin. This is not difficult with hematoxylin, but it seems to us pointless.

Factors Affecting the Permanency of Acid-Fast Stains

When well stained the color of the bacilli in sections should darken slightly within a few days. There is no loss of definition and the color will probably be permanent. We have learned to mistrust the very brightly and brilliantly stained organisms, as they are more likely to fade, for reasons unknown.

If traces of alcohol remain in the mounted section the bacilli may be expected to fade. The ability of alcohol alone slowly to extract the dye explains also why sections must not be left standing in alcohol awaiting mounting, as well as the necessity of using several changes of xylol before mounting. No method is proof against these errors.

An acid balsam or dammar, or one from which the ethereal oils have not been removed (Unna) will bleach the bacilli as it dries.

The best stained and mounted sections will not resist undue amounts of heat which cause the dye to diffuse from the bacilli. Even the temperatures of the tropics may be sufficient to exert a deleterious effect.

Other Staining Reactions of Acid-Fast Bacilli

1. Methylene blue. He who copies Koch's original method of staining acid-fast bacilli with methylene blue will perhaps be surprised by its worth. The bacilli stain readily and well; the selection of a counterstain which affords good contrast and does not affect the color of the bacilli is difficult.

2. Methyl violet, crystal violet, Gram's stain, Much's stain, and so on. Methyl violet was used by Koch and Ehrlich to stain tubercle bacilli before Gram in 1884 devised his method, using the same dye. Acid-fast bacilli are stained by the methylated pararosanilines by either the acid-fast method or Gram's method. The tissues are more difficult to decolorize by the acid-fast method, but the bacilli are better and more permanently colored. Prolonged staining may be required, but acid-fast bacilli appear to be stained much more rapidly by these dyes than by the fuchsins. Lillie's variant of Hucker's formula is preferred to the aniline preparations:

Crystal violet	2 gm.
Alcohol, 95 per cent aqueous	20 cc.
Ammonium oxalate, 1 per cent aqueous to make	100 cc.

We have been unable to distinguish any differences in the color or staining power obtained with crystal violet and the redder methyl violets, which latter probably contain much crystal violet. Hematoxylin, combined with van Gieson's acid fuchsin-picric acid is on the whole preferred to safranine as a counterstain. It is almost certainly true that the wide popular use of fuchsin rather than crystal violet for staining acid-fast bacilli has been due to the want of a wholly adequate contrasting counterstain for the crystal violet, for the latter dye is superior to basic fuchsin in its

staining abilities, although not so much so to new fuchsin in comparable strengths.

3. Thionin. This dye stains acid-fast bacilli, metachromatically, a reddish color. It appears not to be of practical use.

4. Safranine, eosin, neutral red. Safranine has been recommended for staining lepra bacilli in sections. Eosin and neutral red will also stain the bacilli, but the colors of all these dyes are so much less intense and more transparent than that of fuchsin that their use seems uncalled for.

5. Janus green. This dye is probably the equal of fuchsin and crystal violet in its power of staining acid-fast bacilli. The difficulty again is the counterstain.

6. Formaldehyde produces a curious effect on bacilli stained with fuchsin, rendering them violet and highly resistant to decolorization, but not affecting other acid-fast structures, mast cell granules and the shafts of hairs, in the same manner. We have used the following procedure with success, when bacilli were most difficult to stain:

(1) Carbol-new fuchsin 30–60 minutes, or longer.
(2) Formaldehyde, 40 per cent, 5 minutes.
(3) Acid alcohol until nearly decolorized, 15–20 minutes.
(4) Formaldehyde, 40 per cent, a few seconds.
(5) Counterstain in hematoxylin and van Gieson's stain.

7. When sections are treated with potassium permanganate some of the manganese oxides are deposited in the tissues, although the exact substances are not known. These are also deposited in acid-fast bacilli and are less readily removed from the bacilli by oxalic acid than from the tissues. We have seen bacilli fairly well demonstrated thus, although the effect on the tissues is harmful. In a less heavily impregnated state, however, some bacilli stain rapidly and intensely with fuchsin:

(1) Potassium permanganate, 1 per cent aqueous solution, 5–15 minutes.
(2) Carbol-new fuchsin, 5–30 minutes.
(3) Acid alcohol, which will not wholly decolorize the section, 1 minute.
(4) Potassium permanganate, 1 per cent aqueous solution, until the section is a definite brown.

(5) Oxalic acid, 2 per cent aqueous solution.
(6) Counterstain in methylene blue.

The bacillus of rat leprosy appears to be especially readily stained by this procedure, strikingly more so than the tubercle bacillus, human lepra bacillus and some other acid-fast organisms.

Non-Acid-Fast Forms of Acid-Fast Bacilli Occurring in Tissues

We have tried to emphasize that wholly erroneous conclusions may be drawn with regard to the occurrence of acid-fast bacilli in tissues, unless alcoholic fixation and adequate staining are employed.

When bacilli begin to undergo disintegration in the tissues, which usually takes place intracellularly, they stain less readily and may appear fragmented. In some lesions the cytoplasm of the cell may acquire an acid-fast cast, and in some others we have seen cells containing acid-fast granules or droplets, obviously derived from bacilli. But it seems to us that when acid-fast bacilli are destroyed it is never by the process of losing first their acid-fastness. It may be weakened, but ideal staining methods will still bring it out. This is another way of stating that if the Gram stain shows more bacilli than the acid-fast stain, then the fault is with the latter. All acid-fast bacilli are Gram-positive. When they are destroyed in tissues they lose both properties simultaneously.

Résumé

The optimal staining of acid-fast bacilli in tissues involves fixation in an alcoholic medium, removal of mercuric deposits with iodine followed by alcohol and sodium thiosulphate, and staining in a 1 per cent solution of new fuchsin in 5 per cent phenol and 10 per cent methyl alcohol.

If a potassium dichromate fixative is used the period of fixation must be brief and the tissue thoroughly washed, and the section must be treated with potassium permanganate and oxalic acid.

Time and materials are wasted in trying to stain acid-fast bacilli in tissues subjected to prolonged passage in formaldehyde or decalcifying fluids.

To ensure the maximal staining possible under the conditions of fixation, the sections should be stained:

After alcohol fixation, at 20° C. — 2–8 hours,
at 37° C. — 1–4 hours,
at 60° C. — 30 min. to 2 hours, or
at 90° C. (steaming) — 5 minutes;
After all other fixation, at 20° C. — 16–24 hours,
at 37° C. — 12–16 hours,
at 60° C. — 8–12 hours, or
at 90° C. — 5 minutes.

Under good conditions bacilli may be adequately stained in much shorter periods of time than these. Bacilli not stained by fuchsin in the maximum times given will not be stained by further treatment. This is the point to which the duration of staining is best carried. Prolonged staining at room temperature is preferred.

New fuchsin as a dye for acid-fast bacilli is much superior to basic fuchsin.

When, in Zenker fixed tissues, fuchsin fails to stain bacilli resistantly, they may sometimes still be stained by Gram's stain, by the acid-fast method using crystal violet, or by the fuchsin-formaldehyde method here recorded.

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EXSTROPHY OF THE BLADDER WITH IMPERFORATE ANUS, ABSENCE OF THE GREATER PART OF THE SMALL AND LARGE INTESTINES, CONTINUITY OF THE DUODENUM WITH THE COLON, ABSENCE OF THE LEFT TESTIS, EPIDIDYMIS AND CORD, AND ENORMOUS HYDROURETER *

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On May 17th, 1936, a 17 year old colored multipara was admitted to the Maternity Pavilion, Hospital of the University of Pennsylvania, in active labor 2 months before term. Presentation was by the breech, and the birth of a malformed infant was followed by its death in about 10 minutes.

The body was that of a slightly premature male infant 39 cm. in length and 2000 gm. in weight. The head, thorax, back and limbs seemed to be normal, but most of the anterior abdominal wall consisted only of a translucent tough membrane (peritoneum? membrana reuniens?) that extended from the xiphoid and rib margins downward to the pelvis and was pressed forward, like a balloon, about 6 cm. in diameter, by the viscera behind it. There was no umbilicus; the cord, of which a generous portion was still present, divided several centimeters before reaching the body, each half being attached to the outer side of the membranous belly covering, and continuing over it until the skin margin was reached. The branch on the right side contained the umbilical vein and found its way to the liver; that on the left side contained a single artery.

There was complete exstrophy of the bladder, whose posterior wall was so completely everted as to form a dark colored rugose body that not only projected forward but, probably because of the pressure behind, downward as well, until its appearance and position closely resembled an enlarged scrotum. At the anterior, or superior, border of this everted bladder there was a somewhat ragged transverse slit from which a small quantity of greenish paste (meconium?) escaped. At the posterior, or inferior border, there was another less definite slit from which nothing escaped.

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The penis consisted of two short dark colored cylinders, placed side by side, thought to correspond with the corpora cavernosa, and a third, less definite cylinder below and to the right side, terminating in a knob — probably the corpus spongiosum with the glans. Below these structures were two folds of skin projecting laterally to the right and left, with a ridge between, supposed to be the halves of the scrotum and the intervening raphe. No testes were enclosed beneath these folds.

Behind these structures the perineum was entire, smooth, and without the usual dimple to mark the position at which the anus should have been formed.

There were no ureteral orifices on the lower lateral surfaces of the inverted bladder wall. The mucous membrane of the bladder did not meet with skin, but on each side there was an interval of about 4 cm. where the membranous inferior abdominal wall thickened greatly and became tough and elastic. Equidistant from the bladder proper, on each side, this thickened area presented a dark colored eminence of somewhat spongy appearance, as though the summit had been adherent to something that had been torn away. The probable nature of these eminences will later be brought out.

On each side of the rudimentary genitalia there was a hard, slightly elastic, somewhat pointed projection supposed to be the imperfectly formed and widely separated pubic bones.

The malformations thus far described were visible externally and consisted of: (1) a large congenital umbilical hernia; (2) exstrophy of the bladder; and (3) imperforate anus.

As soon as the abdominal cavity was opened a large cystic body at first thought to be a tumor escaped through the incision. It was about 8 cm. in diameter, thin walled and apparently multiloculated; it filled the left side of the abdomen from diaphragm to pelvis. Both kidneys were found in their respective positions and were of normal size and general appearance. The pelvis of the left kidney appeared normal but the ureter, just below the infundibulum, was considerably dilated. With the intention of introducing a probe into the ureter, a snip was made with scissors, when clear fluid escaped under pressure and the largest loculus of the tumor slowly collapsed. It was thus made probable that the supposed cyst was a dilated ureter and that the escaping fluid was urine.

Selecting a cyst at the opposite pole of the tumor, a snip was

made in it and the fluids escaping from both cavities were caught in separate test tubes. Subsequent examination showed both fluids to be urine. As the urine escaped the whole tumor mass gradually collapsed, showing that all of its chambers communicated. By gently drawing upon different parts of the cyst walls and carefully breaking up the external connections that bound the cysts together, it was finally determined that instead of a cyst or cystic tumor, the structure under examination was an enormously dilated convoluted ureter whose approximated twists had been united by external adhesions.

By what mechanism this dilatation had been brought about could not be determined, as a probe passed upward through the first snip made in the ureter entered the pelvis of the kidney without meeting with any obstacle. The pelvis and its infundibulum were normal; it was only the lower part of the ureter that had become enormously dilated.

When all of the adhesions had been separated so that the dilated tortuous structure could be sufficiently straightened, the probe easily found its way from the pelvis of the kidney to the lower termination of the ureter, which was not, however, in an ostium on the bladder wall, as is usually the case in exstrophy of the bladder, but in the spongy tissue immediately subjacent to the eminence of dark colored, thickened surface tissue described above.

The right kidney, its pelvis and its ureter were normal in size and appearance, but a probe introduced into this ureter found its way to the corresponding eminence on the right side and found no point of exit. As, however, this ureter was not dilated, the urine must have escaped through minute openings in the spongy tissue. Both ureters terminated in the same indefinite porous tissue in the respective lateral eminences on the external surface. It seems, therefore, not improbable that the superficial area of thickened membrane with the small polypoid inverted bladder in the middle, and the projecting boss on each side, represented the structures that should have formed the trigone of the bladder, and were largely made up of substance derived from the lower parts of the wolffian ducts.

Deep down behind the other viscera in the right flank, a testis, epididymis and spermatic cord were found. There was no corresponding structure on the left side.

The next striking abnormalities had to do with the alimentary canal.

The stomach appeared to be normally placed in the vertical position and was of about the normal size and shape. Beyond the pyloris was a short tortuous duodenum that continued into a thick walled tube 5 to 6 cm. in length, presenting an appearance closely resembling large intestine. It was almost white in color, and though not more than 5 mm. in diameter, was marked by grooves suggesting the valvulae conniventes. On microscopic section this structure proved to be colon. When a probe was passed from the stomach through the duodenum and on down through this short colon, it escaped through the slit-like opening mentioned above as occurring above the exstrophic bladder and between it and the imperfect abdominal wall — that from which meconium escaped.

The general direction of this short bit of gut was up and down, and beside it, almost in the midline of the abdomen, there was an ambiguous straight structure of cylindrical form whose direction was also directly up and down. At its free upper extremity, which was cecal and rounded, there was a kink, by which the first centimeter of its length was made to assume an obtuse angle with the remainder. The cylinder, almost white in color, was attached by a short straight mesentery. Microscopic sections made through this structure showed it to be perfectly formed small intestine, the mucosa having well formed villi. A probe introduced into its lumen passed upward to the cecal end; passed downward it escaped through the externally visible slit between the exstrophic bladder and the malformed genitalia. There were no other intestines, small or large.

The remaining abdominal viscera seemed to be without interest. The liver, gall-bladder, spleen, adrenals and pancreas were normal in size, shape and position.

The heart and lungs were of normal size, shape and position. There was a large thymus.

To the external malformations previously mentioned must now be added the following internal ones: (4) congenital absence of the greater part of the intestinal canal; (5) communication between the duodenum and the colon; (6) an isolated short length of small intestine opening externally; (7) enormous hydroureter; and (8) absence of left reproductive organs.

Comment

It is difficult to understand by what circumstances these malformations were brought about. It was remarkable that the lower three-fourths of a ureter could be so enormously distended without disturbance of the pelvis of the corresponding kidney and the occurrence of hydronephrosis. The absence of the reproductive organs of the left side might conceivably be the result of pressure atrophy caused by the heavy hydroureter mentioned above. It is difficult to imagine, but it may be possible that originally there had been a complete alimentary canal so disturbed by the pressure of the hydroureter that almost all of it had disappeared, and of the two vestiges of the intestine that remained, an abnormal ulceration of the duodenum into the colon resulted in one piece consisting of stomach, duodenum and ascending colon, and the other in an isolated piece of small intestine. But how did the vestige of the colon come to open externally above the exstrophic bladder and the vestige of the ileum to open externally below it?

The theories explaining the occurrence of exstrophy of the bladder do not seem adequate to explain how the trigone became spread out to the great extent of tissue seen in this case or how it came about that the ureters did not open on the sides of the bladder as usual but terminated in the peculiar spongy tissue described.

DESCRIPTION OF PLATE

PLATE 129

FIG. 1. Gross photograph showing the translucent membranous enclosure of the abdomen, through which the viscera are visible near the center and in the midline below the dark posterior vesical wall rounded and digitate and suggestive of the penis or scrotum.

FIG. 2. Rear view showing the prolapsed membranous belly wall at A, the everted posterior vesical wall at B, and the deformed penis at C. Above the dark penis is a fold of skin covering from side to side just below the coccygeal region, which is the imperfect and empty scrotum, D.

FIG. 3. The exstrophic vesical region viewed from behind and below. A = the membranous abdominal wall; B = the projecting rugose posterior vesical wall; C = the glans of the malformed penis; D = position of the slit-like orifice through which a probe passed into the short piece of small intestine; E = spongy tissue at which the ureters terminated blindly; F = pubic bone widely separated; and G = scrotum.

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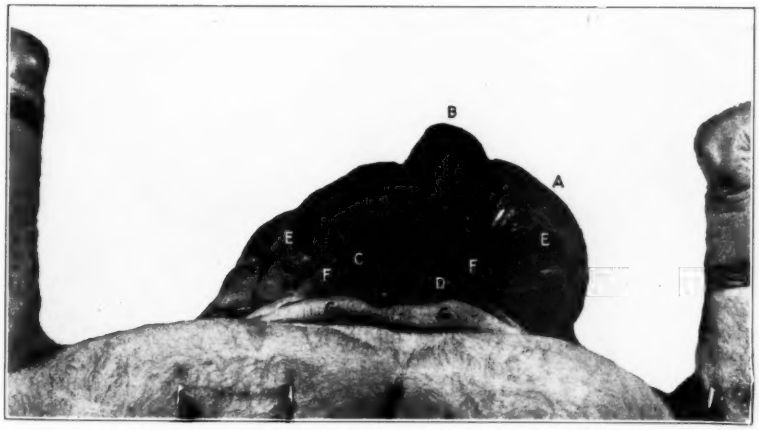
2



3

McFarland Exstrophy of Bladder

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